

# ***European Commission***



**Draft Renewal Assessment Report prepared according to the Commission  
Regulation (EU) N° 1107/2009**

**FLUFENACET**

**Volume 3 – B.7 (AS)**

Rapporteur Member State: Poland  
Co-Rapporteur Member State: France

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### Version History

When	What
August 1997	Initial assessment. <b>Draft Assessment Report</b> for first inclusion to Annex I. RMS: FR
April 2016	<b>Draft Renewal Assessment Report</b> prepared according to the Commission; Regulation (EU) N° 1107/2009; RMS: PL; Co-RMS: FR
May 2017	Revision according to the Co-RMS comments

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**B.7. RESIDUE DATA****INTRODUCTION**

This dossier is submitted to support the re-approval of the active substance Flufenacet in Europe according to Regulation 1107/2009 and the Regulation 844/2012.

Flufenacet was originally included in Annex I of Directive 91/414/EEC on 01/01/2004, as notified in Directive 2003/84/EC dated 25 September 2003 wherein there is no specific provision under Part B which needs to be considered related to the metabolism and residue data.

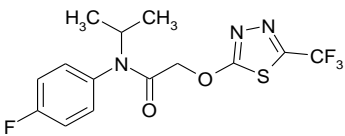
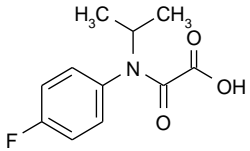
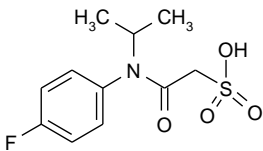
The Monograph prepared by the Rapporteur Member State France in the context of the inclusion of flufenacet in Annex I of the Council Directive 91/414/EEC, the Review Report for flufenacet (7469/VI/98-Final – 3<sup>rd</sup> July 2003) and the EFSA's Reasoned Opinion on the review of existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689) are considered to provide the relevant scientific information for the review of the active substance. Information on the residue definition can be taken from the Complete List of Endpoints, Report of ECCO 73, Annex 2, 5 Residue Section.

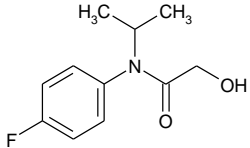
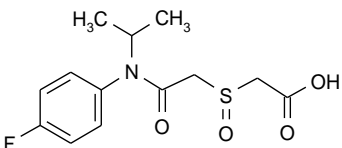
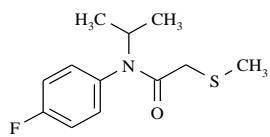
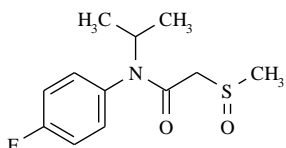
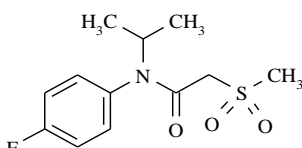
This draft renewal assessment report (DRAR) summaries of studies on flufenacet, which were not available at the time of the Annex I inclusion under Directive 91/414/EEC and were, therefore, not evaluated during the first EU review of this compound. All studies, which were already submitted for the Annex I inclusion under Directive 91/414/EEC are briefly summarized.

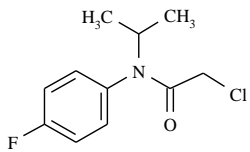
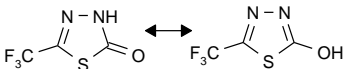
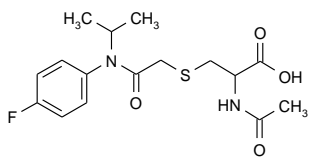
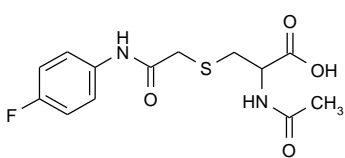
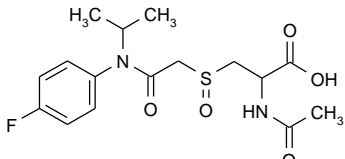
The representative uses supported for the Annex I inclusion process were the outdoor treatment of maize, soya bean, sunflower and cereals (wheat/triticale, rye and barley) at application rates between 0.12 and 0.6 kg a.s./ha in northern and/or southern Europe. In the flufenacet renewal dossier the notifier has included only "representative use" on wheat, barley and rye at application rates between 0.12 and 0.24 kg a.s./ha in northern and southern Europe.

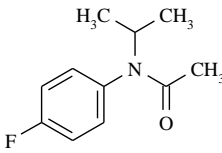
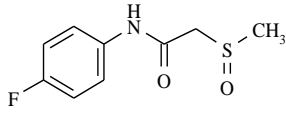
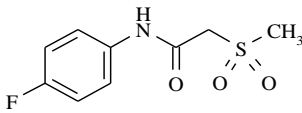
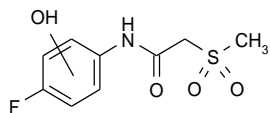
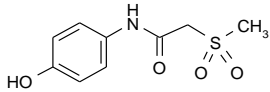
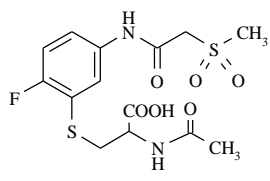
For clarity a list of metabolites, which contains the structures, synonyms, codes and chemical names is presented below in Table 7-1. The matrices in which the metabolites were identified are also included in this list.

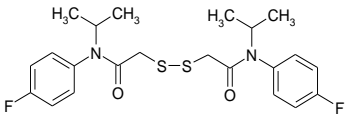
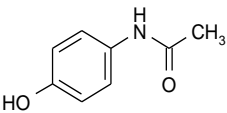
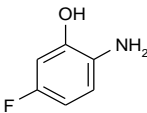
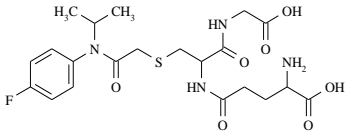
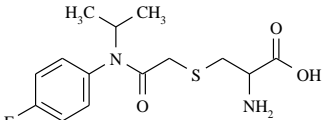
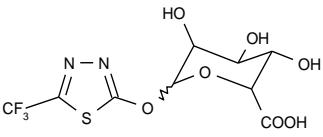
**Table 7-1. Substances and metabolites; structures, codes, synonyms**

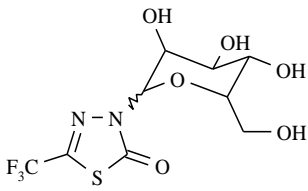
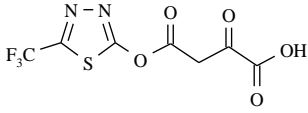
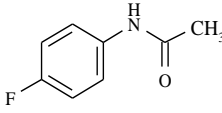
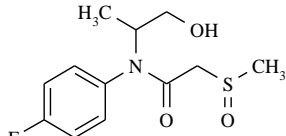
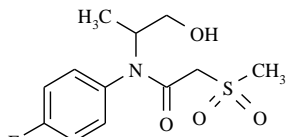
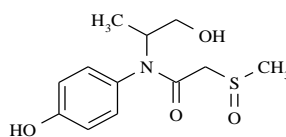
No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
a.s.	 <p>flufenacet (active substance)</p>	<p>FOE 5043, AE F133402, BCS-AB27364</p> <p>IUPAC: 4'-fluoro-N-isopropyl-2-[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yloxy]acetanilide</p> <p>N-(4-fluorophenyl)-N-isopropyl-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide (generated by ICS Naming)</p> <p>CAS: N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide</p>	
M01	 <p>FOE oxalate</p>	<p>FOE5043-oxalate FOEOXALATE, FOEACID OXALATE AE 0841913 BCS-AB16305</p> <p>IUPAC: [(4-fluorophenyl)(isopropyl)amino](oxo)acetic acid (generated by ICS Naming)</p> <p>CAS: Acetic acid, 2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxo-</p>	Goat, hen
			Corn, soybean, cotton, wheat; Rotational crops: kale, turnip, wheat
			Soil (aerobic & anaerobic) Water (aerobic)
M02 <sup>a</sup>	 <p>FOE sulfonic acid</p>	<p>FASO3H AE 0841914 KTS 9465 (sodium salt) BCS-AZ23374 (sodium salt) WAK 6222 (acid) ethanesulfonic acid sodium salt</p> <p>IUPAC: 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethanesulfonic acid sodium 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethanesulfonate (both generated by ICS Naming)</p> <p>CAS: sodium salt: (2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxo-ethanesulfonic acid sodium salt)</p>	Rat
			Corn, soybean, wheat, potato, cotton; Rotational crops: kale, turnip, wheat
			Soil (aerobic & anaerobic) Water (aerobic)

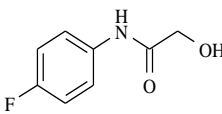
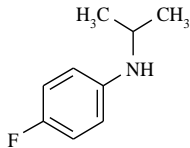
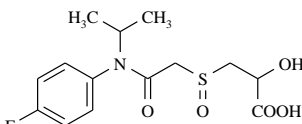
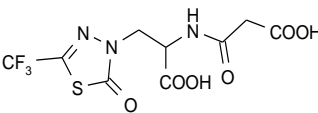
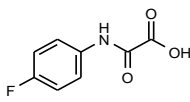
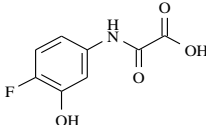
No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M03	 <p>FOE alcohol</p>	<p>FOEALC AND 1403</p> <p>IUPAC: N-(4-fluorophenyl)-2-hydroxy-N-isopropylacetamide (generated by ICS Naming)</p>	Rat
			Corn, soybean, wheat, potato, cotton; Rotational crops: kale, turnip, wheat
			Soil (aerobic & anaerobic) Water (aerobic)
M04	 <p>FOE thioglycolate sulfoxide</p>	<p>FAMSOC TGS FOE mercapto acetic acid sulfoxide AE 0841915 BCS-AB68868</p> <p>IUPAC: ({2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}sulfinyl)acetic acid (generated by ICS Naming)</p>	-
			Corn, soybean, wheat, potato, cotton; Rotational crops: kale, turnip, wheat
			Soil (aerobic) Water (aerobic)
M05	 <p>FOE methylsulfide</p>	<p>WAK 7825 FAMS BCS-CP38571</p> <p>IUPAC: N-(4-fluorophenyl)-N-isopropyl-2-(methylsulfanyl)acetamide (generated by ICS Naming)</p>	Goat, hen
			Corn
			Water (aerobic)
M06	 <p>FOE methylsulfoxide</p>	<p>FOE methyl sulfoxide, FAMSO</p> <p>IUPAC: N-(4-fluorophenyl)-N-isopropyl-2-(methylsulfinyl)acetamide (generated by ICS Naming)</p>	Rat
			Corn, soybean; Rotational crops: kale wheat
			Soil (aerobic) Water (aerobic)
M07	 <p>FOE methylsulfone</p>	<p>FAMSO2 FOE methyl-sulfone BCS-CO62475</p> <p>IUPAC: N-(4-fluorophenyl)-N-isopropyl-2-(methylsulfonyl)acetamide (generated by ICS Naming)</p>	Rat
			Corn, soybean; Rotational crops: kale wheat
			Soil (aerobic) Water (aerobic)

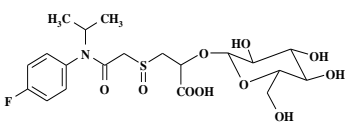
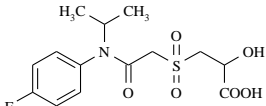
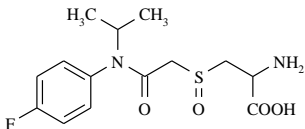
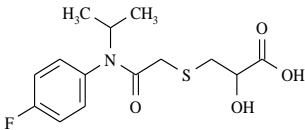
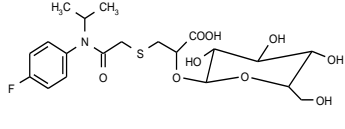
No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M08	 <p>FOE chloroacetanilide</p>	BCS-AA70824  IUPAC: 2-chloro-N-(4-fluorophenyl)-N-isopropylacetamide (generated by ICS Naming)	Rat
			Corn, soybean;  Rotational crops: kale wheat
			Soil (aerobic)
M09	 <p>FOE-thiadone (keto-enol tautomers)</p>	Thiadone TH HWH 4343 BCS-AA41715  IUPAC: 5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-one, 5-(trifluoromethyl)-1,3,4-thiadiazol-2-ol (generated by ICS Naming)	Rat (detected as aglycon and as glucuronide (M24) and oxalyl-acetate conjugates (M26); Goat, hen
			Corn, soybean;  Rotational crops: transient metabolite as it is detected as N- glucoside (M25) and N- malonyl-alanine conjugate (M34)
			Soil (aerobic & anaerobic) Water (aerobic)
M 10	 <p>FOE acetyl cysteine</p>	FANACS FOE-mercapturic acid  IUPAC: N-acetyl-S-{2-[(4-fluorophenyl) (isopropyl)amino]-2-oxoethyl}cysteine (generated by ICS Naming)	Rat, goat, hen
			-
			-
M 11	 <p>FOE des-i-propyl cysteine</p>	DIFANACS  IUPAC: N-acetyl-S-{2-[(4-fluorophenyl)amino]-2-oxoethyl}cysteine (generated by ICS Naming)	Rat
			-
			-
M 12	 <p>FOE S-oxo cysteine</p>	FANACSO  IUPAC: N-acetyl-3-({2-[(4-fluorophenyl) (isopropyl)amino]-2-oxoethyl}sulfinyl)alanine (generated by ICS Naming)	Rat
			-
			-

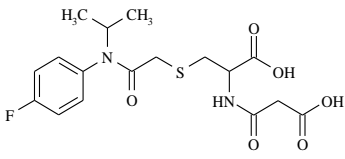
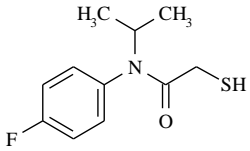
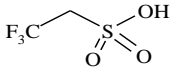
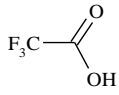
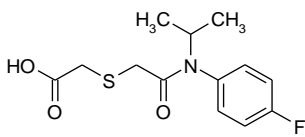
No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M 13	 <p>FOE amine acetate</p>	FAAC  IUPAC: N-(4-fluorophenyl)-N-isopropylacetamide (generated by ICS Naming)	Rat Hen
			Corn
			-
M 14	 <p>FOE des-i-propyl methylsulfoxide</p>	DIFAMSO BCS-AH21407  IUPAC: N-(4-fluorophenyl)-2-(methylsulfinyl) acetamide (generated by ICS Naming)	Rat (transient)
			-
			-
M 15	 <p>FOE des-i-propyl methylsulfone</p>	DIFAMSO2  IUPAC: N-(4-fluorophenyl)-2-(methylsulfonyl) acetamide (generated by ICS Naming)	Rat, Goat (following feeding of parent flufenacet. However, the parent substance is not present in ruminant feed)
			-
			-
M 16	 <p>fluoro-hydroxy-des-i-propyl methylsulfone</p>	HODIFAMSO2  (no exact structure defined)	Rat
			-
			-
M 17	 <p>hydroxy-des-i-propyl methylsulfone</p>	HODIFAMSO2  IUPAC: N-(4-hydroxyphenyl)-2-(methylsulfonyl) acetamide (generated by ICS Naming)	Rat
			-
			-
M 18	 <p>hydroxy-des-i-propyl methylsulfone-glutaminic acid</p>	HODIFAMSO2-Glu  IUPAC: N-acetyl-S-(2-fluoro-5-[(methylsulfonyl) acetyl]amino}phenyl)cysteine (generated by ICS Naming)	Rat
			-
			-

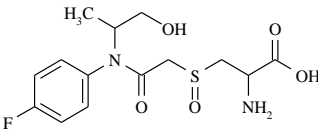
No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M 19	 <p>FOE disulfide</p>	FOE-thiol dimer BCS-BJ39504  IUPAC: 2,2'-disulfanediylbis[N-(4-fluorophenyl)-N-isopropylacetamide] (generated by ICS Naming)	Rat
			-
			-
M 20	 <p>hydroxy-des-i-propyl amine acetate</p>	DIFANAc DIFAOAc (regio-isomers), BCS-AF93293  IUPAC: N-(4-hydroxyphenyl)acetamide (generated by ICS Naming)	Rat
			-
			-
M 21	 <p>2-A-5-FP</p>	2-amino-5-fluorophenol BCS-AA53294  IUPAC: 2-amino-5-fluorophenol (generated by ICS Naming)	Rat
			-
			-
M 22	 <p>FOE glutathione</p>	FOEGSH  IUPAC: gamma-glutamyl-S-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteinylglycine (generated by ICS Naming)	Rat, goat and hen: Primary transient metabolite
			Plants: Primary transient metabolite of the main metabolic pathway
			-
M 23	 <p>FOE cysteine</p>	FOE cysteinyl conjugate FACS  IUPAC: S-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteine (generated by ICS Naming)	Rat, goat and hen: Transient secondary metabolite
			Plants: Transient secondary metabolite of the main metabolic pathway, detected in potato tuber
			-
M 24	 <p>thiadone glucuronide</p>	Th glucuronide TH-GA  IUPAC: 5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl hexopyranosiduronic acid (generated by ICS Naming)	Rat, goat, hen
			-
			-

No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M 25	 <p>ThN glucoside</p>	<p>THNG Th-NG Thiadone-N-glucoside</p> <p>IUPAC: 3-hexopyranosyl-5-(trifluoromethyl)-2,3-dihydro-1,3,4-thiadiazol-2-one (generated by ICS Naming)</p>	Soybean, corn
			Rotational crops: kale, turnip, wheat
			-
M 26	 <p>Th oxalyl acetic acid</p>	<p>Th-OAA</p> <p>IUPAC: 2,4-dioxo-4-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]butanoic acid (generated by ICS Naming)</p>	Rat
			-
			-
M 27	 <p>DIFAAC</p>	<p>N-(4-Fluorophenyl) acetamide BCS-AA22989</p> <p>IUPAC: N-(4-fluorophenyl)acetamide (generated by ICS Naming)</p>	Hen (following feeding of parent flufenacet; not relevant as the parent substance is not present in poultry feed)
			-
			-
M 28	 <p>HOIFAMSO</p>	<p>IUPAC: N-(4-fluorophenyl)-N-(1-hydroxypropan-2-yl)-2-(methylsulfinyl)acetamide (generated by ICS Naming)</p>	Hen (following feeding of parent flufenacet; not relevant as the parent substance is not present in poultry feed)
			-
			-
M 29	 <p>HOIFAMSO2</p>	<p>IUPAC: N-(4-fluorophenyl)-N-(1-hydroxypropan-2-yl)-2-(methylsulfonyl)acetamide (generated by ICS Naming)</p>	Rat Hen (following feeding of parent flufenacet; not relevant as the parent substance is not present in poultry feed)
			-
			-
M 30	 <p>LMeOH-3</p>	<p>IUPAC: N-(4-hydroxyphenyl)-N-(1-hydroxypropan-2-yl)-2-(methylsulfinyl)acetamide (generated by ICS Naming)</p>	Hen
			-
			-

No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M 31	 Des-isopropyl-FOE alcohol	DIFOEALC BCS-AB35517  IUPAC: N-(4-fluorophenyl)-2-hydroxy-N-acetamide;  N-(4-fluorophenyl)-2-hydroxyacetamide (generated by ICS Naming)	Rat
			-
			-
M 32	 4-Fluoro-N-(1-methylethyl)benzamine	FA, BCS-AA57901 AE F145057  IUPAC: 4-fluoro-N-isopropylaniline (generated by ICS Naming)	Rat
			-
			-
M 33	 FOE sulfinyl lactic acid	FAMSOL FAMSOL-I FAMSOL-II (diastereomeric pair)  IUPAC: 3-( { 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl } sulfinyl )-2-hydroxypropanoic acid (generated by ICS Naming)	-
			Corn, Wheat; Rotational crops: kale, turnip, wheat
			-
M 34	 Th-malonylalanyl conjugate	THNMALALA  IUPAC: N-(carboxyacetyl)-3-[2-oxo-5-(trifluoromethyl)-1,3,4-thiadiazol-3(2H)-yl] alanine (generated by ICS Naming)	-
			Soybean
			-
M 35	 FOE des-isopropyl oxalate	IUPAC: [(4-fluorophenyl)amino](oxo)acetic acid (generated by ICS Naming)	-
			Rotational crops: turnip, wheat
			-
M 36	 FOE 3-OH-des-isopropyl oxalate	IUPAC: [(4-fluoro-3-hydroxyphenyl)amino](oxo)acetic acid (generated by ICS Naming)	-
			Rotational crops
			-

No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M 37	 <p>FOE sulfinyl lactic acid glucoside</p>	<p>FAMSOL-Glu FAMSOL-Glu-I FAMSOL-Glu-II</p> <p>IUPAC: 3-({ 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl }sulfinyl)-2-(hexopyranosyloxy) propanoic acid (generated by ICS Naming)</p>	-
			Corn, Wheat; Rotational crop: wheat
			-
M 38	 <p>FOE sulfonyl lactic acid</p>	<p>IUPAC: 3-({ 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl }sulfinyl)-2-(hexopyranosyloxy) propanoic acid (generated by ICS Naming)</p>	-
			Rotational crops: turnip, wheat
			-
M 39	 <p>FOE cysteine sulfoxide</p>	<p>FACSO</p> <p>IUPAC: 3-({ 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl }sulfinyl)alanine (generated by ICS Naming)</p>	Fish
			Rotational crops: kale, wheat
			-
M 40 <sup>b</sup>	 <p>FOE sulfanyl lactic acid</p>	<p>FAMSL</p> <p>IUPAC: 3-({ 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl }sulfanyl)-2-hydroxypropanoic acid (generated by ICS Naming)</p>	-
			Wheat, potato (transient: glucoside conjugate formed)
			-
M 41 <sup>b</sup>	 <p>FOE sulfanyl lactic acid glucoside</p>	<p>FAMSL-Gl, FAMSL-Glu</p> <p>IUPAC: 3-({ 2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl }sulfanyl)-2-(hexopyranosyloxy) propanoic acid (generated by ICS Naming)</p>	-
			Potato, wheat
			-

No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M 42	 <p>FOE malonylcysteine conjugate</p>	<p>FAM-MalCys</p> <p>IUPAC: N-(carboxyacetyl)-S-{2-[(4-fluorophenyl)(isopropyl)amino]-2-oxoethyl}cysteine (generated by ICS Naming)</p>	-
			Corn
			-
M 43	 <p>FOE free sulfide</p>	<p>IUPAC: N-(4-fluorophenyl)-N-isopropyl-2-sulfanylacetyl (generated by ICS Naming)</p>	Goat, hen
			Corn; Rotational crops
			-
M44 <sup>a</sup>	 <p>FOE 5043-trifluoroethanesulfonic acid</p>	<p>TFESA BCS-CU62474 (sodium salt)</p> <p>IUPAC: 2,2,2-trifluoroethanesulfonic acid</p>	-
			-
			Soil (aerobic & anaerobic)
M45 <sup>a</sup>	 <p>trifluoroacetic acid</p>	<p>TFA AE C502988 (acid) AE1046319 (sodium salt) BCS-AZ56567 (sodium salt)</p> <p>IUPAC: trifluoroacetic acid</p>	Rat
			Rotational crops: turnip, Swiss chard, wheat (main metabolite in all rotated crops)
			Soil (aerobic & anaerobic)
M46	 <p>FOE thioglycolate sulfide</p>	<p>FOE thioglycolate</p> <p>IUPAC: 4-Fluoro-N-methylethylaniline thiodiacetic acid amide</p>	-
			-
			Soil (anaerobic) Water (aerobic)

No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Occurrence
M47	 <p>isopropyl hydroxy cysteine</p>	<p>FAIOCS</p> <p>IUPAC: 3-({ 2-[(4-fluorophenyl)(1-hydroxypropan-2-yl)amino]-2-oxoethyl}sulfinyl)alanine (generated by ICS Naming)</p>	<p>Fish</p> <p>-</p> <p>-</p>
<p><sup>a</sup> The structures and report names of degradation products/metabolites identified in e-fate and metabolism studies reflect in general their uncharged species. The degradation products/metabolites FOE sulfonic acid, FOE 5043-trifluoroethanesulfonic acid and trifluoroacetic acid have pKa-values &lt; 2 and hence, are deprotonated under environmental and physiological conditions and show no acidic properties anymore, due to the high buffer capacities of the environmental and physiological matrices. Therefore, their environmental and physiological relevant deprotonated species (i.e. their alkali salts) were used for all studies which were conducted to elucidate the toxicological and ecotoxicological properties of these degradation products/ metabolites as well as their fate in the environment, plants and animals.</p> <p><sup>b</sup> In the first Annex I Listing process the metabolite numbers M40 and M41 were allocated to carbon dioxide and methane. However these two compounds are not considered as unique metabolites of flufenacet but as common mineralization products. Hence, these two metabolite numbers were reallocated to two metabolites identified in metabolism studies (FOE sulfanyl lactic acid and FOE sulfanyl lactic acid glucoside).</p>			

### B.7.1. STORAGE STABILITY OF RESIDUES

#### Plant Matrices

Storage stability data was reported in chapter 6 of the Annex II dossier (Bosnak, L. L.; 1997; M-002426-01). The freezer storage stability of flufenacet (FOE 5043) and five of its metabolites (FOE-oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide, FOE methylsulfoxide, and FOE methylsulfone) was examined in commodities of three different crops, representing oil-, starch- and water containing materials. Field grown corn grain, forages, and fodder; soybean seeds, forage, and hay; and turnip roots and tops were fortified at a nominal rate of 1 mg/kg with the radiolabeled compounds. The first study covers a storage period of 11 months for all commodities. In the addendum, freezer storage stability data for turnips up to 20 months and for corn and soybean commodities up to 28 months were reported. The results show that residues of flufenacet and its metabolites are stable in all tested matrices under frozen conditions for at least as long as the storage stability studies lasted. Storage stability data were considered appropriate in the Monograph (Annex B 6) and in the EFSA Reasoned Opinion on existing MRLs (EFSA Journal 2012;10(4): 2689). Thus, in principle, no further data is required. The data already evaluated is briefly summarised in Table 7.1-1.

**Table 7.1-1: Maximum demonstrated storage stability for flufenacet and metabolites in plant matrices**

Compound	Commodity	Matrix type	Maximum storage period (months) AII 6.3.3/01	Maximum storage period (months) AII 6.3.3/02	Storage conditions	Reference
Flufenacet, FOE-oxalate, FOE-sulfonic acid, FOE-thioglycolate sulfoxide, FOE-methylsulfoxide, FOE-methylsulfone	Corn grain	High starch content	11	28	≤ -21°C	KCA 6.1/01  Monograph Annex B 6  EFSA Reasoned Opinion 2012
	Corn forage	High water content	11	28		
	Corn fodder	High water content	11	28		
	Soybean seed	High oil content	11	28		
	Soybean forage	High water content	11	28		
	Soybean hay	Dry	11	28		
	Turnip roots	High starch content	11	20		
	Turnip tops	High water content	11	20		

Additional storage stability information is reported in a study from the US on wheat commodities (wheat forage, grain and straw) for flufenacet and the five metabolites mentioned above for storage periods up to 21 months. The study was not evaluated during the EU peer review and in principle no additional data were considered necessary relative to the uses evaluated for Annex I listing. For sake of completeness the study on wheat commodities is summarised below since it may provide supplementary information relative to the representative use on cereals.

In addition data were generated to demonstrate storage stability for additional commodity groups of high protein content (dry bean seed) and high acid content (orange fruit) as outlined in OECD guideline 506 (stability of pesticide residues in stored commodities). The study is also summarized below.

<b>Report:</b>	<b>KCA 6.1/04</b> , Bosnak, L. L.; 1997; M-002424-01
<b>Title:</b>	The storage stability of FOE 5043 and metabolites in wheat forage, grain, and straw
<b>Report No. &amp; Document No.:</b>	107137 dated April 22, 1997 M-002424-01-1
<b>Guidelines:</b>	Fulfils data requirement of US EPA 171-4(e) Storage Stability - Crops
<b>GLP:</b>	Yes; Deviations: none

## Material and Methods

Freezer storage stability of flufenacet (FOE 5043) and five of its metabolites (FOE-oxalate, FOE sulfonic acid, FOE thioglycolate sulfoxide, FOE methylsulfoxide, and FOE methylsulfone) was examined in commodities of wheat (grain, straw and forage). The study was performed using [fluorophenyl-UL-<sup>14</sup>C]flufenacet and metabolites with the <sup>14</sup>C label in the fluorophenyl ring. Sample materials were fortified with different treating solutions:

- i) Solution A containing 1.01 ppm flufenacet + 1.02 ppm FOE thioglycolate sulfoxide (FAMSOC) in flufenacet equivalents
- ii) Solution B containing 0.99 ppm FOE oxalate + 1.10 ppm FOE methylsulfoxide (FAMSO) in flufenacet equivalents
- iii) Solution C containing 1.02 ppm FOE sulfonic acid (FASO3H) + 1.02 ppm FOE methylsulfone (FAMSO2) in flufenacet equivalents.

**Fortification:** 10 g samples of pulverized and frozen wheat forage, grain, and straw were weighed into glass jars. The jars were closed, labeled, and placed in frozen storage (-24±5°C) until fortification. Three unfortified samples of each matrix were designated as blank controls. The samples for spiking were removed from the freezer, allowed to warm to room temperature, and were fortified with 1 mL of solution A, B or C, nominally at 1 mg/kg for each analyte. The samples were manually shaken and rotated to distribute the fortification solution on the matrix. Three of the samples of each matrix/fortification solution combination were selected for immediate (zero-time) extraction and analysis; the remaining samples were returned to frozen storage.

**Sample extraction:** At each sampling interval, replicate fortified samples (triplicate at zero time and duplicate at 6 and 21 months) of each matrix were extracted by repeated blending with methanol for 2 to 3 minutes and filtered. The filtered extracts were combined, radioassayed by LSC, and analyzed by HPLC. **Analytical methodology:** Liquid samples were radioassayed by LSC. Aliquots of solid samples were combusted using a sample oxidizer and the resulting <sup>14</sup>CO<sub>2</sub> was trapped in alkaline solution and measured by LSC. HPLC analyses were conducted on a C8 column preceded by a reverse-phase precolumn and radioactivity was quantified using a radioactivity monitor. The peaks observed during HPLC analysis of the extracts corresponded to the peaks for the compounds fortified in the samples.

To verify the identity of each peak, extracts representing each dosing solution and each matrix were spiked with standards for each analyte and subjected to co-chromatography by HPLC. The corresponding standard for each analyte was shown to co-elute with the identified peak.

## Findings

The recoveries of flufenacet and the five metabolites in the extract for each time point are given in Tables 7.1-2 to 7.1-7. The analytical method was suitable for determining residues in the storage stability study. Samples were fortified with [<sup>14</sup>C]-labeled analytes, and analyses were performed by HPLC. Peak identification was verified by co-chromatography with known standards of each analyte. Recoveries of radioactivity from the HPLC column at each storage interval were as follows: 92-103% at time-zero, 89-107% at 6 months, and 91-122% at 21-months. After 21 months of storage, recoveries of flufenacet-related residues ranged from 84 to 120% (calculated as percent of measured time-zero residue) for wheat forage, grain, and straw fortified with each analyte at ~1 mg/kg.

## Conclusion

Under freezer conditions -24±5°C flufenacet and five of its metabolites (FOE-oxalate, FOE sulfonic acid,

FOE thioglycolate sulfoxide, FOE methylsulfoxide, and FOE methylsulfone) were found to be stable for at least 21 months in wheat forage, grain and straw. No significant degradation was observed for any of the analytes after 21 months. After 21 months of storage, recoveries of flufenacet-related residues (calculated as percent of measured time-zero residue) ranged from 84 to 120% for wheat forage, grain, and straw fortified with each analyte at ~1 mg/kg.

**Table 7.1-2: Storage stability of [<sup>14</sup>C]flufenacet in wheat commodities**

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery <sup>1</sup>
Forage	1.02	0	0.93; 0.96; 0.94	0.94	--
	1.02	6	0.92; 0.96;	0.94	100
	1.02	21	0.99; 0.89	0.94	100
Grain	1.02	0	0.89; 0.88; 0.86	0.88	--
	1.02	6	0.83; 0.86	0.84	95
	1.02	21	0.74; 0.74	0.74	84
Straw	1.02	0	0.98; 0.94; 0.93	0.95	--
	1.02	6	0.79; 0.75	0.77	81
	1.02	21	0.86; 0.83	0.84	88

<sup>1</sup> % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

**Table 7.1-3: Storage stability of [<sup>14</sup>C] FOE thioglycolate sulfoxide (FAMSOC) in wheat commodities**

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery <sup>1</sup>
Forage	1.01	0	1.16; 1.19; 1.29	1.21	--
	1.01	6	1.24; 1.20	1.22	101
	1.01	21	1.05; 1.25	1.15	95
Grain	1.01	0	1.02; 0.90; 0.94	0.95	--
	1.01	6	0.84; 0.83	0.84	88
	1.01	21	1.17; 1.10	1.14	120
Straw	1.01	0	1.00; 1.04; 0.98	1.01	--
	1.01	6	0.77; 0.72	0.74	73
	1.01	21	1.17; 1.12	1.14	113

<sup>1</sup> % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

**Table 7.1-4: Storage stability of [<sup>14</sup>C] FOE oxalate in wheat commodities**

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery <sup>1</sup>
Forage	0.99	0	1.01; 0.96; 0.91	0.96	--
	0.99	6	0.99; 1.00	1.00	104
	0.99	21	0.96; 0.95	0.96	100
Grain	0.99	0	0.88; 0.86; 0.80	0.85	--
	0.99	6	0.68; 0.77	0.72	85
	0.99	21	0.72; 0.74	0.73	86
Straw	0.99	0	0.93; 0.87; 0.95	0.92	--
	0.99	6	0.71; 0.69	0.70	76
	0.99	21	0.88; 0.88	0.88	96

<sup>1</sup> % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

**Table 7.1-5: Storage stability of [<sup>14</sup>C] FOE methylsulfoxide (FAMSO) in wheat commodities**

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery <sup>1</sup>
Forage	1.10	0	1.12; 1.11; 1.10	1.11	--
	1.10	6	1.06; 0.99	1.02	92
	1.10	21	1.16; 1.06	1.11	100
Grain	1.10	0	1.06; 0.99; 1.01	1.02	--
	1.10	6	0.85; 0.93	0.89	87
	1.10	21	0.95; 0.93	0.94	92
Straw	1.10	0	1.02; 0.97; 1.06	1.02	--
	1.10	6	0.82; 0.77	0.80	78
	1.10	21	0.99; 1.02	1.0	98

<sup>1</sup> % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

**Table 7.1-6: Storage stability of [<sup>14</sup>C] FOE sulfonic acid (FASO3H) in wheat commodities**

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery <sup>1</sup>
Forage	1.02	0	1.06; 1.07; 1.08	1.07	--
	1.02	6	1.00; 1.06	1.03	96
	1.02	21	1.16; 1.14	1.15	107
Grain	1.02	0	0.90; 0.89; 0.89	0.89	--
	1.02	6	0.85; 0.89	0.87	98
	1.02	21	0.94; 0.89	0.92	103
Straw	1.02	0	0.92; 0.89; 0.94	0.92	--
	1.02	6	0.79; 0.83	0.81	88
	1.02	21	1.04; 1.05	1.04	113

<sup>1</sup> % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

**Table 7.1-7: Storage stability of [<sup>14</sup>C] FOE methylsulfone (FAMSO2) in wheat commodities**

Sample material	Spike level (mg/kg)	Storage interval (months)	Recoveries in extract (mg/kg)	Mean (mg/kg)	% apparent stored recovery <sup>1</sup>
Forage	1.02	0	0.98; 1.03; 1.04	1.02	--
	1.02	6	0.90; 0.98	0.94	92
	1.02	21	0.88; 0.93	0.90	88
Grain	1.02	0	0.93; 0.96; 0.97	0.95	--
	1.02	6	0.75; 0.82	0.78	82
	1.02	21	0.81; 0.82	0.82	86
Straw	1.02	0	0.96; 0.97; 1.02	0.98	--
	1.02	6	0.85; 0.90	0.88	90
	1.02	21	0.89; 0.86	0.88	90

<sup>1</sup> % Apparent stored recovery = (Recovered residue after storage/Recovered residue at time-zero) x 100. Values calculated using the average recovered residue at each storage interval. No concurrent recoveries were determined for the stored samples. Therefore, no corrections were made based on concurrent recovery values.

<b>Report:</b>	<b>KCA 6.1/02</b> , Stuke, S.; Ballmann, C.; 2013; M-439517-02_
<b>Title:</b>	Amendment no. 1 to report no: P642100741 - Storage stability of flufenacet and metabolites in/on orange fruit and dry bean seeds for 24 months
<b>Report No. &amp; Document No.:</b>	MR-10/006, dated October 08, 2012 ; amended 2013-11-05 M-439517-02-1
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>– Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances (reference to document no. 7032/VI/95 rev.5 Appendix H)</li> <li>– US EPA Residue Chemistry Test Guideline OCSP 860.1380: Storage Stability Data</li> <li>– OECD Test Guideline 506, adopted 16 October 2007</li> <li>– PMRA Ref.: DACO 7.3, Storage Stability</li> </ul>
<b>GLP:</b>	Yes; Deviations: none

## Material and Methods

To determine the freezer storage stability of flufenacet (FOE 5043) and its metabolites in plant materials, individual 5-g control samples of orange fruit (high acid content) and bean seed (high protein content) were spiked with parent flufenacet or a 1/1/1 mixture of its metabolites FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid, separately, resulting in a fortification level of either 0.10 mg/kg of flufenacet or the metabolite mixture. All fortification levels are expressed as parent equivalents. Except for the day-0 analysis, samples were stored in amber glass bottles in a deep-freezer at -18°C or below for later use. For day-0 analysis, five spiked samples of each sample material and two blank control samples were analysed. In addition, two concurrent recoveries spiked at the respective LOQ level were performed. Further samples were also analysed after nominal storage intervals of 1 (only dry bean seed), 2 (only orange fruit), 6, 12 and 24 months (both commodities). At each of these intervals, three treated samples and three control samples of each material were removed from storage and analysed. Two control samples were fortified for the determination of concurrent recoveries. Samples used for concurrent recoveries were fortified freshly on the day of analysis at the same magnitude as the spiked storage samples.

The total residue of flufenacet (flufenacet and its metabolites containing the *N*-fluorophenyl-*N*-isopropyl amine moiety) in/on matrices of plant origin was analytically determined as 4-fluoro-*N*-isopropylaniline using analytical method 01100 by LC-MS/MS (Billian, P.; 2010; M-362575-02). The LOQ is 0.01 mg/kg expressed as flufenacet equivalents.

## Findings

Data on procedural recoveries are summarized in Tables 7.1-8 and 7.1-9. Storage stability data for flufenacet and the metabolite mix are summarized in Table 7.1-10 to 7.1-13.

Mean procedural recoveries analysed alongside with the stored samples were within the range of 81-119% for both matrices for the parent compound (overall at 0.1 mg/kg 87-97 % per matrix). For the metabolite mix, procedural recoveries ranged from 61- 99 % for both sample materials and all storage intervals (overall mean 77-85 %). RSDs were always below 20%. Residues in the control samples were below 30% of the LOQ for each storage interval and both matrices.

After a deep-freezer storage period of about 24 months, the mean recovery rate for flufenacet from the stored samples of orange fruit was 98 % (111 % normalized to day 0). In samples of dry bean seed the mean recovery was 87 % (99 % normalized to day 0). After the longest storage period of 24 months, recoveries fortified with the metabolite mix (FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid) were 68 % (94% normalized to day 0) and 71% (108% normalized to day 0) in orange fruit and dry bean seed, respectively. Recoveries of the metabolite mix were generally lower for both, the stored samples and the freshly fortified samples, compared to the parent compound. However, normalized to the recoveries at day 0 it is evident that the lower values do not indicate any degradation.

## Conclusion

The study results demonstrate stability of flufenacet and the representative metabolites FOE oxalate, FOE thioglycolate sulfoxide and FOE sulfonic acid containing the *N*-fluorophenyl-*N*-isopropyl amine moiety for at least 24 months in frozen storage at  $\leq -18$  °C in the tested plant commodities (dry bean seed, orange fruit) representing the commodity groups of high protein content and high acid content.

**Table 7.1-8: Concurrent Recoveries for Flufenacet (FOE 5043)**

Sample Material	Nominal Storage Interval [d]	Concurrent Recoveries [%]			
		0.01 mg/kg fort. level		0.10 mg/kg fort. level	
		Single Values	Mean	Single Values	Mean
Orange fruit	0	118, 71	95	-	-
	60	-	-	91, 88	90
	180	-	-	85, 94	90
	360	-	-	85, 90	88
	720	-	-	81, 80	81
Overall mean, RSD		Overall mean = 95		Overall mean = 87, RSD = 5.6	
Dry bean seed	0	106, 101	104	-	-
	30	-	-	105, 132	119
	180	-	-	106, 101	104
	360	-	-	87, 80	84
	720	-	-	90, 76	83
Overall mean, RSD		Overall mean = 104		Overall mean = 97, RSD = 18.5	

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

**Table 7.1-9: Concurrent Recoveries for Analyte Mixture of FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid**

Sample Material	Nominal Storage Interval [d]	Concurrent Recoveries [%]			
		0.01 mg/kg fort. level		0.10 mg/kg fort. level	
		Single Values	Mean	Single Values	Mean
Orange fruit	0	72, 70	71	-	-
	60	-	-	76, 74	75
	180	-	-	94, 98	96
	360	-	-	72, 78	75
	720	-	-	59, 63	61
Overall mean, RSD		Overall mean = 71		Overall mean = 77, RSD = 17.7	
Dry bean seed	0	69, 73	71	-	-
	30	-	-	86, 85	86
	180	-	-	95, 103	99
	360	-	-	73, 93	83
	720	-	-	66, 78	72
Overall mean, RSD		Overall mean = 71		Overall mean = 85, RSD = 14.4	

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

**Table 7.1-10: Storage stability data for Flufenacet (FOE 5043) in orange fruit**

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Orange Fruit	Flufenacet (FOE 5043)						
	0	0.087 0.090 0.094 0.090 0.079	87 90 94 90 79	88	100	95	93
	60	0.096 0.091 0.070	96 91 70	86	98	90	96
	180	0.104 0.102 0.098	104 102 98	101	115	90	112
	360	0.081 0.094 0.099	81 94 99	91	103	88	103
	720	0.089 0.101 0.103	89 101 103	98	111	81	121

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

<sup>a</sup> Normalized Recovery = (Average recovery / average recovery at day 0) X 100%

<sup>b</sup> Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

**Table 7.1-11: Storage stability data for analyte mixture of FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid in orange fruit**

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Orange Fruit	Metabolite mix: FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid						
	0	0.074	74	72	100	71	101
		0.067	67				
		0.074	74				
		0.070	70				
		0.073	73				
	60	0.077	77	76	106	75	101
		0.075	75				
		0.075	75				
	180	0.082	82	86	120	96	90
		0.089	89				
		0.086	86				
	360	0.070	70	69	96	75	92
		0.068	68				
0.068		68					
720	0.066	66	68	94	61	111	
	0.073	73					
	0.065	65					

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

<sup>a</sup> Normalized Recovery = (Average recovery / average recovery at day 0) X 100%

<sup>b</sup> Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

**Table 7.1-12: Storage stability data for Flufenacet (FOE 5043) in dry bean seed**

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Dry bean seed	Flufenacet (FOE 5043)						
	0	0.071	71	88	100	104	85
		0.097	97				
		0.082	82				
		0.091	91				
		0.098	98				
	30	0.109	109	104	119	119	87
		0.094	94				
		0.109	109				
	180	0.094	94	101	115	104	97
		0.101	101				
		0.107	107				
	360	0.073	73	79	90	84	94
		0.084	84				
		0.080	80				
	720	0.087	87	87	99	83	105
		0.100	100				
		0.074	74				

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

<sup>a</sup> Normalized Recovery = (Average recovery / average recovery at day 0) X 100%

<sup>b</sup> Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

**Table 7.1-13 Storage stability data for analyte mixture of FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid in dry bean seed**

Commodity	Nominal Storage Period (days)	Residue Level in Stored Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Dry bean seed	Metabolite mix: FOE oxalate hydrate, FOE thioglycolate sulfoxide, FOE sulfonic acid						
	0	0.075	75	66	100	71	93
		0.067	67				
		0.073	73				
		0.056	56				
		0.059	59				
	30	0.078	78	74	112	86	86
		0.065	65				
		0.078	78				
	180	0.094	94	96	145	99	97
		0.095	95				
		0.098	98				
	360	0.069	69	71	108	83	86
		0.073	73				
		0.070	70				
	720	0.070	70	71	108	72	99
		0.064	64				
		0.079	79				

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

<sup>a</sup> Normalized Recovery = (Average recovery / average recovery at day 0) X 100%

<sup>b</sup> Corrected percent recovery = (Average % recovery (stored) / Average of fresh concurrent recoveries) X 100%

In three trials from residue studies 12-2001 and 12-2002 from 2012 for some field samples the requested storage temperature of -18°C was exceeded due to problems during shipment (Table 7.1-14). In order to address this deviation a short term storage stability study was conducted. The storage conditions tested were such that the most unfavorable conditions which were determined for all shipments are covered.

**Table 7.1-14: Deviations in conditions of storage temperature for field samples**

Study number	Trial number	Maximum temperature reached	Total duration above -18°C	Average temperature above -18°C
12-2001	12-2001-01	-10°C	08 h, 10 m	-14.8°C
12-2002	12-2002-03	1°C	4 d 04 h 15 m	-13.9°C
12-2002	12-2002-03	-0.5°C	3 d 17 h 15 m	-11.6°C
12-2002	12-2002-04	-5.6°C	6 d, 15 h, 00 m	-15.8°C

d = day, h = hour, m = minutes

<b>Report:</b>	<b>KCA 6.1/03</b> , Klimmek, S.; Gizler, A.; 2013; M-467724-02-1
<b>Title:</b>	7 days freezer storage stability study of flufenacet (FOE5043) and its metabolites in tomato and wheat grain
<b>Report No. &amp; Document No.:</b>	S13-02753, dated 2013-10-08, amended 2013-11-19 M-467724-02-1
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>– Commission Regulation (EU) No 544/2011 of 10 June 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the data requirements for active substances</li> <li>– US EPA Residue Chemistry Test Guideline OPPTS 860.1380: Storage Stability Data</li> <li>– OECD Test Guideline 506, adopted 16 October 2007</li> <li>– PMRA Ref.: DACO 7.3, Storage Stability</li> </ul>
<b>GLP:</b>	Yes, deviations: none

## Material and Methods

The objective of the study was to evaluate the stability of flufenacet (FOE 5043) and its metabolites after storage for a period of 4 hours at +1°C followed by 7 days at -10°C in tomato and wheat grain as representatives for two different commodity groups.

Individual aliquots of plant material from tomato and wheat grain were fortified with 1.0 mg/kg of a mixture of flufenacet (FOE 5043) and its metabolites FOE oxalate hydrate, FOE sulfonic acid (as Na salt) and FOE thioglycolate sulfoxide (3/1/1/1). The samples were stored in plastic containers at an average temperature of +1°C for 4 hours and at -10°C for the following 7 days and were analysed at the nominal storage intervals of 0 and 7 days.

On day 0, for each matrix, six samples were prepared with 5 g of specimen material. Then, five containers were fortified with a mixture of Flufenacet (FOE 5043) and its metabolites FOE oxalate hydrate, FOE sulfonic acid (as Na salt) and FOE thioglycolate sulfoxide (3/1/1/1) at 1.0 mg/kg and one was used without fortification as a control specimen. The samples were analysed directly.

For analysis at day 7, for each matrix, eight samples were prepared with 5 g of specimen material. Five containers were fortified with a mixture of Flufenacet (FOE 5043) and its metabolites FOE oxalate hydrate, FOE sulfonic acid (as Na salt) and FOE thioglycolate sulfoxide (3/1/1/1) at 1.0 mg/kg. Three containers were stored without fortification to be used as control material and procedural recoveries. The storage containers were placed in a freezer at +1(±0.5)°C immediately after the fortification. After 4 hours the storage containers were placed in a freezer at -10°C for seven days. The temperature of the freezers was continually recorded with a data recorder.

The five freshly fortified tomato and wheat grain specimen fortified at 1.0 mg/kg on day 0 also served as method validation recoveries. Two concurrent recoveries were conducted at 1.0 mg/kg in tomato and wheat grain, at 7 days of storage.

The total residue of flufenacet (flufenacet and its metabolites containing the *N*-fluorophenyl-*N*-isopropyl amine moiety) in/on matrices of plant origin was analytically determined as 4-fluoro-*N*-isopropylaniline using analytical Method 01100/M002 (Stuke, S.; Teubner, L.; 2013; M-448503-01).

Samples were extracted under acidic and oxidative conditions. After steam distillation of the common

moiety 4-fluoro-*N*-isopropylaniline, samples were analysed with high performance liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) using an internal standard for quantification.

## Findings

The recoveries in the freshly fortified samples proved the method performance. Mean recoveries for the amount of total residues of flufenacet ranged between 71% and 84%. Two mass transitions were monitored and provided comparable results.

In addition, 2 concurrent recoveries per commodity were conducted at the nominal storage intervals of 7 days. Recoveries were at 70% and 85% for tomato and wheat grain, respectively. Validation and procedural recoveries are summarised in Table 7.1-15.

In the control samples of tomato and wheat grain, total residues of flufenacet were below the LOQ (0.01 mg/kg).

The recoveries of the stored samples showed that the total residue of flufenacet, determined as 4-fluoro-*N*-isopropylaniline, is stable in plant matrices (tomato and wheat grain) for at least 4 hours at +1°C followed by 7 days at -10°C. After 7 days of storage, recoveries were 71% for tomato and 82% for wheat grain (normalised to day 0: 99% and 98% for tomato and wheat grain, respectively). Table 7.1-16 summarises the total residues of flufenacet in tomato and wheat grain stored spiked samples, as well as the corresponding mean concurrent recovery data.

## Conclusion

The findings from short-term storage stability study demonstrate that the temperature deviations during shipment did not result in a negative impact on the quality of the residue studies concerned.

The storage conditions tested were such that the most unfavorable conditions which were determined for all shipments are covered. Residues of flufenacet proved to be stable under the experimental conditions tested.

**Table 7.1-15: Procedural recovery data for the total residue of flufenacet**

Plant Material	Fortification Level [mg/kg]	Date of Extraction	Storage Interval (days)	Flufenacet (FOE5043) and metabolites Single Recoveries [%]					Mean	RSD	Standard Deviation
									[%]	[%]	[%]
Tomato	1.0	2013-07-04	0	74	68	72	72	71	71	3.1	2.2
	1.0	2013-07-11	7	79	61	-	-	-	70	-	-
	Overall Mean, RSD and standard deviation [%]								71	7.8	5.5
Wheat Grain	1.0	2013-07-04	0	80	75	100	85	79	84	12	9.7
	1.0	2013-07-11	7	82	87	-	-	-	85	-	-
	Overall Mean, RSD and standard deviation [%]								84	9.6	8.1

RSD: relative standard deviation

**Table 7.1-16: Storage stability data for flufenacet in tomato and wheat grain**

Commodity	Storage Period (days)	Residue Level in Stored Spiked Samples			Day-0 Normalized Recovery <sup>a</sup>	Average % of Fresh Concurrent Recoveries	Average Corrected % Recovery <sup>b</sup>
		mg/kg (ppm)	% of nominal spiking level	Average % recovery			
Tomato	0	0.740	74	71	100	NA	NA
		0.680	68				
		0.720	72				
		0.720	72				
		0.710	71				
	7	0.700	70	71	99	70	101
		0.710	71				
		0.700	70				
		0.730	73				
		0.700	70				
Wheat Grain	0	0.800	80	84	100	NA	NA
		0.750	75				
		1.000	100				
		0.850	85				
		0.790	79				
	7	0.890	89	82	98	85	97
		0.750	75				
		0.770	77				
		0.880	88				
		0.830	83				

determined as 4-fluoro-*N*-isopropylaniline, calculated and expressed as flufenacet

<sup>a</sup>Normalized Recovery = (Average recovery / average recovery at day 0) X 100%

<sup>b</sup>Corrected percent recovery = (Average % recovery (stored spiked sample) / Average of fresh concurrent recoveries) X 100%

NA = Not applicable

## Animal Matrices

No additional studies were provided.

Analysis of the goat matrices (Monograph, France, 1997) showed, that FOE oxalate was stable in goat tissues and milk for 540 to 650 days at  $24 \pm 1^\circ\text{C}$  (Freeseaman, P-L., Jett, C.M., Minor, R.G.; 1995). Based on above, it was assumed that the compound is stable in cow tissues and milk as well. Samples from the cow feeding study were stored concurrently with the samples from the [ $^{14}\text{C}$ ]FOE oxalate goat metabolism study in the same freezer and under the same storage conditions (Duah, F. K.; 1995).

Based on the metabolism data from lactating goat and laying hen using [fluorophenyl- $^{14}\text{C}$ ]flufenacet, [phenyl- $^{14}\text{C}$ ]FOE oxalate - which is the main plant metabolite- and [thiadiazole-2- $^{14}\text{C}$ ]flufenacet it was concluded in the DAR/Monograph that residues are unlikely to occur in tissues or products of farm animals.

Actually feeding studies were not triggered but a 29-day cattle feeding study had been conducted for US EPA and was submitted with the first dossier (Duah, 1995; M-002268-01-1). The cattle feeding study had been performed using FOE oxalate as a predominant plant metabolite since the parent compound itself was not detected in plant matrices. Since feeding studies using non-radio labelled active substance actually were not triggered according to the EU data requirements the need to evaluate storage stability data for animal commodities did not arise.

The evaluation of the magnitude of residues in livestock from EFSA confirms the conclusions drawn in the

EU review process (EFSA Reasoned Opinion on existing MRLs, EFSA Journal 2012:10(4):2689):

*“On the basis of the animal metabolism studies it is concluded that, after exposure to the maximum dietary burden (about 200 times lower than the dose level in the metabolism studies [ie. 5 mg/kg bw/d]; ...), residue levels in livestock commodities are expected to remain below the enforcement LOQ of 0.01 mg/kg in milk, 0.02 mg/kg in liver and 0.05 mg/kg in fat, eggs, kidney and muscle. Hence, no livestock feeding study is needed; MRLs and risk assessment values for the relevant commodities in ruminants, pigs and poultry can be established at the LOQ level.” (p.29/30).*

It is therefore concluded that there would be still no need to investigate the storage stability of flufenacet residues in animal commodities.

## Summary

In the EU review process (Directive 91/414/EEC) storage stability data were evaluated for flufenacet and five metabolites (FOE-oxalate, FOE-sulfonic acid, FOE-thioglycolate sulfoxide, FOE-methylsulfoxide, FOE-methylsulfone) in matrices of corn, soybean (up to 28 months) and turnips (20 months) covering the commodity groups of high water content, high oil content and high starch content.

In the supplementary dossier additional storage stability information is provided on wheat commodities (wheat forage, grain and straw) for flufenacet and the five metabolites for up to 21 months and for additional commodity groups of high protein content (dry bean seed) and high acid content (orange fruit) for up to 24 months (flufenacet, FOE-oxalate, FOE-sulfonic acid, FOE-thioglycolate sulfoxide).

No significant decrease of residues was observed for flufenacet and its metabolites after the tested periods. Thus the residues of flufenacet and its metabolites are stable in plant matrices under freezer storage conditions for at least as long as the storage periods lasted.

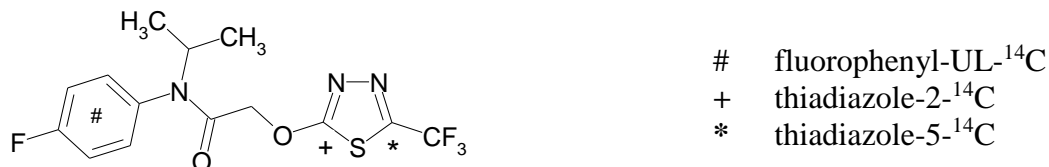
In addition, in some samples of supportive trials from three residue studies the requested temperature of -18°C was exceeded due to problems during the shipment of these samples. In order to address this deviation, a short-term storage stability study was conducted (tomato and wheat grain as representatives for two different commodity groups). Residues of flufenacet proved to be stable under the experimental conditions tested reflecting the conditions during shipment.

Analysis of the goat matrices in feeding study (Monograph, France, 1997) showed, that FOE oxalate was stable in goat tissues and milk for 540 to 650 days. No additional studies were provided.

## B.7.2. METABOLISM, DISTRIBUTION AND EXPRESSION OF RESIDUES

### B 7.2.1 Plants

Flufenacet was  $^{14}\text{C}$ -labelled at three different positions of the molecule for investigation of metabolism studies in plants and animals:



Most of the plant metabolism studies were conducted with [fluorophenyl-UL- $^{14}\text{C}$ ]flufenacet. These studies included maize/corn, soybeans and cotton (all pre-planting treatment) as well as the rotated crops kale, turnip and wheat with different plant back intervals. For soybeans (pre-planting treatment) and the rotated crops the [thiadiazole-2- $^{14}\text{C}$ ] label was used additionally. These studies were submitted with the dossier for Annex I listing of flufenacet according to EU directive 91/414/EEC and reported in the Tier 2 summary for the active substance, under Annex IIA, Point 6.1 (1996). As a consequence, they were already evaluated during the Annex I listing process and considered appropriate to describe the metabolism in plants.

They were reconsidered for renewal of active substance approval as well.

The initial metabolic reaction is cleavage of the molecule into the thiadone and acetamide moiety. While the resulting thiadone (M09) itself was not observed, various conjugates were formed, the most important being the corresponding *N*-glucoside (M25). In soybeans, the malonylalanine conjugate (M34) predominated.

The fluorophenyl-acetamide portion is directly conjugated with glutathione (GSH) or homoglutathione (hGSH) and further metabolized yielding the transient FOE cysteine conjugate (M23). All subsequent metabolites can be considered as hydrolysis, oxidation and conjugation products of the glutathione pathway. However, the FOE oxalate (M01) most likely arose through direct oxidation of the transient hydrolysis product of flufenacet, the primary alcohol (FOE alc, M03).

#### Residue definition for food of plant origin

From these studies a conclusion on the residue definition in food of plant origin was made: “*The metabolism of the flufenacet results in a number of metabolites, which all have the common moiety N-isopropyl-4-fluorophenyl. Although no parent compound was found in any study and only three metabolites were of quantitative significance (M01: FOE oxalate; M02: FOE sulfonic acid, M04: FOE thioglycolate sulfoxide) a “total residue” approach is proposed, based on the total amount of N-fluorophenyl-N-isopropyl derived residues.*” (Monograph on FOE 5043 (flufenacet), Annex B.6, Section B.6.3).

This approach (only when application is made pre- and early post-emergence) has been confirmed by EFSA in “Reasoned opinion on the review of the existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005”, EFSA Journal 2012;10(4):2689. Consequently the currently binding residue definition for food of plant origin (for monitoring and enforcement purposes) according to Commission Regulation (EU) No 1127/2014 of 20 October 2014 is “**Flufenacet (sum of all compounds containing the N-fluorophenyl-N-isopropyl moiety expressed as flufenacet)**”. Residue definition for risk assessment purposes is proposed in the EFSA opinion to be the same. If in the future uses are needed where the application is closer to harvest the metabolism and residue definition would need to be reconsidered.

Additional plant metabolism studies were conducted later which were not included in the original Annex II dossier and thus not evaluated by a peer review on the EU level. These are studies of [fluorophenyl-UL- $^{14}\text{C}$ ]flufenacet on potato (pre-planting and post-emerging treatment) and on wheat and maize (both post-emerging treatment). The studies were submitted and evaluated in different EU Member States in support of uses in potatoes and maize. They will now also be reported in this summary.

To complete the knowledge on the metabolic pathway in plants additional metabolism studies were conducted on wheat (post-emerging treatment), potato (pre-emerging treatment) and on the rotated crops turnip, Swiss chard and wheat. All of these later studies were conducted with flufenacet radiolabeled in the [thiadiazole-5-<sup>14</sup>C] position. Finally, [thiadiazole-5-<sup>14</sup>C]flufenacet was also used in a supporting metabolism study in the rat.

An overview of all plant metabolism studies of radiolabeled flufenacet and the different positions of the <sup>14</sup>C-label is compiled in Table 7.2.1-1.

**Table 7.2.1-1: Overview of all plant metabolism studies with <sup>14</sup>C-labeled flufenacet in primary crops**

Study type	Crop	Application scenario	Label	Report	Submission	
					EU baseline dossier, Annex II , Section 4, Point 6	Reported in supplementary dossier Section 6.2
Plant metabolism	Corn (maize)	Pre-emergence application	[Fluorophenyl-UL- <sup>14</sup> C]	Baird, J. H.; 1994; M-002270-01-1	KCA 6.2.1/01	-
	Soybean	Pre-emergence application	[Fluorophenyl-UL- <sup>14</sup> C] [Thiadiazole-2- <sup>14</sup> C]	Krolski, M. E.; Bosnak, L. L.; 1995; M-002278-01-1	KCA 6.2.1/02	-
	Cotton	Pre-emergence application	[Fluorophenyl-UL- <sup>14</sup> C]	Krolski, M. E.; Bosnak, L. L.; 1995; M-002277-01-1	KCA 6.2.1/03	-
	Plant cell suspension cultures	--	[Fluorophenyl-UL- <sup>14</sup> C] [Thiadiazole-2- <sup>14</sup> C]	Koester, J.; Brauner, A.; 1995; M-002366-01-1	KCA 6.2.1/04	--
	Potato	Pre- and post-emergence application	[Fluorophenyl-UL- <sup>14</sup> C]	Beedle, E. C.; Ying, S. L.; 2000; M-020428-01-1		KCA 6.2.1/07
	Wheat	Post emergence application	[Fluorophenyl-UL- <sup>14</sup> C]	Krolski, M. E.; Bosnak, L. L.; 1997; M-002275-01		KCA 6.2.1/05
	Corn (maize)	Post emergence application	[Fluorophenyl-UL- <sup>14</sup> C]	Krolski, M. E.; Bosnak, L. L.; 1998; M-005755-01		KCA 6.2.1/06
	Wheat	Post emergence application	[Thiadiazole-5- <sup>14</sup> C]	Bongartz, R.; Miebach, D.; 2013; M-444475-01-1		KCA 6.2.1/09
	Potato	Pre-emergence application	[Thiadiazole-5- <sup>14</sup> C]	Bongartz, R.; 2012; M-441506-02-1		KCA 6.2.1/08

### B 7.2.1.1 Plant metabolism studies evaluated during Annex I submission and reconsidered for renewal of active substance approval

Metabolism of flufenacet was investigated for pre-emergence treatment (via incorporation to soil) on cereals (maize) and pulses & oilseeds (cotton and soya bean), using fluorophenyl-U-<sup>14</sup>C labelled flufenacet. A study for pre-emergence treatment (via incorporation to soil) on pulses and oilseeds (soya bean) was also conducted, using thiadiazole-2-<sup>14</sup>C labelled flufenacet (France, 1997). Details of the studies are given below.

#### Corn

<b>Report:</b>	Baird, J.H., Metabolism of [fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in corn. Bayer Corporation, unpublished report no. MR105027 of 19.12.1994
<b>Guideline:</b>	EPA 171-4(a) Nature of the Residue in Plants
<b>GLP:</b>	yes (certified laboratory)

In review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689) EFSA concluded above study:

*“In the pre-emergence study (evaluated in the DAR) on maize, the highest TRR was seen in fodder (0.498 mg eq./kg). In maize kernels (fresh and dry), residues were too low for identification (0.009 mg eq./kg and 0.012 mg eq./kg respectively). The highest residue levels were found in forage and fodder, where flufenacet oxalate accounted for up to 44 % of the TRR. Flufenacet thioglycolate sulfoxide and flufenacet sulfinyl lactic acid were also found in forage and fodder accounting for up to 11 % and 10 % of the TRR, respectively, all other components were below 10 % of the TRR. Parent flufenacet was completely degraded and not found in either forage or fodder.”*

Description of this study evaluated by RMS (France) is cited below.

*[Fluorophenyl-UL-<sup>14</sup>C]FOE 5043 with a specific radioactivity of 60.0 mCi/mMole (radiochemical purity >98 %) was incorporated into the top 6 cm layer of a sandy loam at a rate of 1370 g a.i./ha (1.81 mg a.i./kg soil), equivalent to 2.28x the highest recommended field application rate in Europe. Corn was planted in buckets containing the treated soil and grown outside or later on in the greenhouse. Samples were taken at day 96 (forage and fresh kernels) and day 110 (fodder and dry kernels).*

*The results of the analyses of the different crop parts are given in table 6.1.1 [current Table 7.2.1.1-1]. At harvest, forage and fresh kernels contained a total radioactive residue (TRR) of 0.261 and 0.009 mg/kg, and fodder and dry kernels of 0.498 and 0.012 mg/kg parent compound equivalents, respectively. About 90 % of the radioactivity in forage and fodder and about 70 % in the kernels could be extracted.*

*The major radioactive component identified in forage and fodder was FOE oxalate (M 1, 44 % and 41 %). Further metabolites were FOE thioglycolate sulfoxide (M 4, 10 % and 11 %), FOE sulfinyl lactic acid (M 33, 10 % and 9 %) and FOE methylsulfoxide (M 7, 4 % and 3 %). In addition, FOE sulfonic acid (M 2, 7% and 5 %) and FOE methylsulfoxide (M 6, <1 % and 1 %) were tentatively identified by comparison of retention times with samples from the soybean study which is described below. Parent compound was not detected in either forage or fodder. In total, 86 % (forage) and 80 % (fodder) of the TRR was identified and no single unidentified metabolite exceeded 7 % of the TRR.*

*Due to the very low levels of radioactivity, even when using an exaggerated treatment rate, no metabolite identification was possible in the kernels.*

**Table 7.2.1.1-1 [Table 6.1.1 in DAR]. Distribution of metabolites (% of recovered radioactivity) in different crop parts of corn after pre-emergence application of [fluorophenyl-UL-<sup>14</sup>C] FOE 5043 to a sandy loam**  
 Application rate: 1370 g a.i./ha

Crop Part	forage	fresh kernels	fodder	dry kernels
Days after Application	96	96	110	110
TRR (mg/kg)	0.261	0.009	0.498	0.012
a.i.	-	-	-	-
M 1	44	-	41	-
M 2	7 ❶	-	5 ❶	-
M 4	10	-	11	-
M 6	<1 ❶	-	1 ❶	-
M 7	4	-	3	-
M 33	10	-	9	-
Remainder ❷	4	42	7	39
Unknown (%)	10	29	13	33
Not Extract. (%)	11	29	10	28
Total	100	100	100	100

❶: tentatively identified by comparison of retention times with samples from the soybean study

❷: fractions not further analysed due to low amounts of radioactivity

M1: FOE oxalate

M2: FOE sulfonic acid

M4: FOE thioglycolate sulfoxide

M6: FOE methylsulfoxide

M7: FOE methylsulfone

M 33: FOE sulfinyl lactic acid

## Conclusion

For the purpose of renewal of a.s. approval, this study was considered as supportive information. In this study no residues have been identified in corn kernels. It can be replaced by the study of Krolski and Bosnak (1997) conducted with the same plant species.

## Cotton

<b>Report:</b>	Krolski, J., Bosnak, L.L. The metabolism of [fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in cotton, Bayer Corporation, report no.: MR106666 of 01.12.1995b
<b>Guideline:</b>	EPA 171-4(a) Nature of the Residue in Plants
<b>GLP:</b>	yes (certified laboratory)

In review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689) EFSA concluded above study:

*“In cotton the highest TRR was identified in forage harvested at 21 days (1.64 mg eq./kg). High levels were also seen in the mature plant (1.54 mg eq./kg). In the seeds, residues were too low for identification (0.067 mg eq./kg). Flufenacet oxalate was the most important component of the residue in forage (29 % TRR) and flufenacet sulfonic acid was the main component of the TRR in the mature plant, accounting for 66 % of the TRR. All other metabolites in all crop parts were below 10 % of the TRR and parent flufenacet was completely degraded and not found at all.”*

Description of this study evaluated by RMS (France) is cited below.

*Cotton seeds were planted in buckets containing a sandy loam and were then covered with soil treated with [Fluorophenyl-UL-<sup>14</sup>C]FOE 5043 with a specific radioactivity of 60.6 mCi/mMole (radiochemical purity > 96 %) at a rate of 2.37 mg a.i./kg soil corresponding to 1778 g a.i./ha. The plants were grown in the greenhouse. Samples were taken 21 days (first immature harvest), 43 days (second immature harvest) and*

156 days after planting (final harvest). At the final harvest cotton lint with seeds was removed from the open bolls and the seeds were then manually removed from the lint. Afterwards, the mature plants were separated into foliage and bolls.

Table 6.1.2 [current Table 7.2.1.1-2] gives the results of the analyses of the different crop parts. At day 21 and day 43 after planting the total radioactive residues were 1.64 and 0.90 mg/kg parent compound equivalents, respectively. At the final harvest the TRR was 1.54 and 0.067 mg/kg in the mature plants and seeds, respectively.

A total of 84 to 88 % of the TRR in the plants could be extracted with MeOH, whereas acid and base treatment of the mature plants increased the extractability to 98 %. In the seeds only 13 % were extracted with MeOH, but acid and base treatment released additional 71 % of the TRR.

The distribution of metabolites in the different crop parts is also given in table 6.1.2 [current Table 7.2.1.1-2]. No active ingredient was identified in any cotton matrix. The major radioactive component identified was FOE sulfonic acid (M 2, 20 % - 66 %). Further metabolites were FOE oxalate (M1, 11-29 %), FOE thioglycolate sulfoxide (M 4, 6 - 7 %) and FOE methylsulfoxide (M 6, 5 - 6 %) and FOE methylsulfone (M 7, 2 - 4 %). In total, 64 % to 85 % of the total radioactive residue was identified and no single unidentified metabolite exceeded 9 % of the TRR.

Due to the very low levels of radioactivity, even when using an exaggerated treatment rate, no metabolite identification was possible in the seeds.

**Table 7.2.1.1-2 [Table 6.1.2 in DAR]. Distribution of metabolites (% of recovered radioactivity) in different crop parts of cotton after pre-emergence application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 to a sandy loam**  
Application rate: 1778 g a.i./ha

Crop Part	forage	forage	mature plant	seeds
Days after Application	21	43	156	156
TRR (mg/kg)	1.64	0.90	1.54	0.067
a.i.	-	-	-	-
M 1	29	22	11	-
M 2	20	43	66	-
M 4	6	7	6	-
M 6	5	6	-	-
M 7	4	2	2	-
M 33	-	-	-	-
Remainder ❶	-	-	6	83
Unknown (%)	22	9	7	-
Not Extract. (%)	14 ❷	12 ❷	2	16
Total	100	100	>98	>99

❶: fractions not further analysed due to low amounts of radioactivity

❷: without acid or base treatment

M1: FOE oxalate

M2: FOE sulfonic acid

M4: FOE thioglycolate sulfoxide

M6: FOE methylsulfoxide

M7: FOE methylsulfone

M 33: FOE sulfinyl lactic acid

## Conclusion

For the purpose of renewal of a.s. approval, this study was considered as acceptable.

## Soybean

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<b>Report:</b>	Krolski, M.E., Bosnak, L.L., Metabolism of FOE 5043 in soybeans. Bayer Corporation report no.: MR105187 of 07.03.1995a
<b>Guideline:</b>	EPA 171-4(a) Nature of the Residue in Plants
<b>GLP:</b>	yes (certified laboratory)

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In review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689) EFSA concluded above study:

*“In soya bean (fluoro-phenyl- $U$ - $^{14}C$  labelled study) the highest TRR was identified in hay (21.73 mg eq./kg) and dry beans at final harvest (1.02 mg eq./kg). In hay and forage three metabolites (flufenacet oxalate, flufenacet sulfonic acid and flufenacet thioglycolate sulfoxide) were present at similar levels and together accounted for 74 – 88 % TRR. Flufenacet thioglycolate sulfoxide was the main component of the TRR in beans, accounting for 26 % of the TRR in beans (80 days) whereas other metabolites were below 10 % of the TRR. Parent flufenacet was completely degraded and not found in any of the samples taken at any time.*

*In soya bean (thiadiazole-2- $^{14}C$  labelled study) the highest TRR seen in the early samples was in forage (2.60 mg eq./kg); and in general the levels of TRR were comparable to those in the fluorophenyl labelled study. In the later samples the highest TRR was identified in hay (5.78 mg eq./kg) and in general the levels of TRR were lower, compared to the fluorophenyl labelled study. The only identified component of the TRR in 21 and 48 day forage was an N-glucoside conjugate of thiaodone19, accounting for 68 % and 61 % of TRR, respectively; the remaining TRR was either unextracted or could not be identified. The N-glucoside conjugate of thiaodone was also the most important component of the residue in 91 day forage and hay (58 % and 66 % TRR, respectively) but a group of four minor metabolites characterised as thiaodone conjugates were also present at levels up to 19 % (total of the four conjugated metabolites). The only known component of the TRR in 91 day beans was tentatively identified as an N-malonylalanine conjugate of thiaodone20, accounting for 66 % of TRR; the remaining fractions were not analysed due to low amounts of radioactivity. Parent flufenacet was completely degraded and not found in any of the samples taken at any time.*

*As fluorine is present in both rings of the flufenacet molecule,  $^{19}F$  NMR was also used to analyse all of the metabolites. It was confirmed, using this technique, that five metabolites containing the fluorophenyl moiety of the molecule were present in all crops (flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide, flufenacet methylsulfoxide and flufenacet methylsulfone21).  $^{19}F$  NMR was also used to compare extracts from the crops treated with fluoro-phenyl- $U$ - $^{14}C$  and soya bean extracts from the study using thiadiazole-2- $^{14}C$ ; the results indicated the metabolic profile was broadly similar in both cases.”*

Description of this study evaluated by RMS (France) is cited below.

*[Fluorophenyl- $UL$ - $^{14}C$ ]FOE 5043 with a specific radioactivity of 60.6 mCi/mMole (radiochemical purity > 98 %) was incorporated into the top 5 cm layer of a sandy loam at a rate of 1485 g a.i./ha (1.98 mg a.i./kg) soil, equivalent to 2.48x the highest recommended field application rate in Europe. Soybean seeds were planted in buckets containing the treated soil and grown in the greenhouse. Samples were taken at day 20 after planting (whole plants), day 42 and 66 (forage and beans) and at day 80 (hay and beans).*

*In a second experiment soybean seeds were planted in buckets containing a sandy loam arid were then covered with soil treated with [thiadiazole-2- $^{14}C$ ]FOE 5043 with a specific radioactivity of 18.47 mCi/mMole (radiochemical purity 98 %) at a rate of 1.84 mg a.i./kg soil, corresponding to 1380 g a.i./ha, which is 2.3 times the highest recommended application rate in Europe. The plants were placed outside and were returned to the greenhouse about two weeks before the final harvest. Samples were taken on day 21 after seeding (whole plants), day 48 (forage and beans), day 91 (forage and beans) and on day 105 (hay).*

*Table 6.1.3a [current Table 7.2.1.1-3] gives the results of the analyses of the different crop parts for the fluorophenyl-experiment. Whole immature soybean plants collected as forage 20 days after planting had an average TRR. of 2.20 mg/kg parent compound equivalents. The corresponding value for forage from immature plants sampled 42 days after planting was 6.82 mg/kg, whereas only 0.17 mg/kg was measured*

in the beans. At day 66 the plants contained a total radioactive residue of 8.49 mg/kg in forage and 0.48 mg/kg in the beans. At day 80, as a consequence of concentration during the drying process, these values increased to 21.73 mg/kg and 1.02 mg/kg, respectively.

At the early sampling dates where no acid and base hydrolyses were performed, 5 to 15 % of the TRR was not extracted. More rigorous extraction procedures of the day 66 and day 80 samples released > 99 % of the TRR.

In total five metabolites were identified and were identical to those found in corn FOE oxalate (M1, 6 % - 26%), FOE sulfonic acid (M2, 5 % - 48 %), FOE thioglycolate sulfoxide (M4, 16 % - 49 %), FOE methylsulfoxide (M6, 2 % - 9 %) and FOE methylsulfone (M7, 3 % - 9 %). The parent compound was not detected. In addition to the radioactivity, which was not further analysed due to low amounts of radioactivity in the corresponding fractions (= remainder), a total of 5 % - 28 % of the recovered radioactivity could not be identified, but was characterised based on extractability and partitioning characteristics. No single metabolite exceeded 7 % of the recovered radioactivity, except in the early samplings, where individual metabolites reached 18 % (day 20, whole plants) or 10 % and 15 % (day 42 beans) of the TRR. However, taking into consideration the low TRR and/or the exaggerated dose rates, it can be concluded that they are of no significance.

**Table 7.2.1.1-3 [Table 6.1.3a in DAR]. Distribution of metabolites (% of recovered radioactivity) in different crop parts of soybeans after pre-emergence application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 to a sandy loam**  
Application rate: 1485 g a.i./ha

Crop Part	whole plant	forage	beans	forage	beans	hay	beans
Days after Application	20	42	42	66	66	80	80
TRR (mg/kg)	2.20	6.82	0.17	8.49	0.48	21.73	1.02
a.i.	-	-	-	-	-	-	-
M 1	15	26	8	18	6	19	6
M 2	19	48	6	42	7	38	5
M 4	16	10	49	17	43	17	26
M 6	9	5	6	6	2	2	6
M 7	6	3	3	9	5	5	4
M 33	-	-	-	-	-	-	-
Remainder ❶	-	-	-	2	17	2	36
Unknown (%)	21	2	28	5	14	13	6
Not Extract. (%)	10 ❷	5 ❷	15 ❷	<1	<1	<1	<1
Total ❸	96	99	115	99	94	96	89

❶: fractions not further analysed due to low amounts of radioactivity

❷: without acid or base treatment

❸: Total does not equal to 100 % due to rounding

M1: FOE oxalate

M2: FOE sulfonic acid

M4: FOE thioglycolate sulfoxide

M6: FOE methylsulfoxide

M7: FOE methylsulfone

M 33: FOE sulfinyl lactic acid

Table 6.1.3b [current Table 7.2.1.1-4] gives the results of the analyses of the different crop parts for the thiadiazole experiment. At day 21 after planting the total radioactive residues were 2.60 mg/kg parent compound equivalents for forage, at day 48 they were 1.23 mg/kg and 0.21 mg/kg for forage and beans, respectively. These amounts at the early sampling dates are comparable to those found in the study using fluorophenyl labelled active ingredient. However, the total radioactive residues at the later sampling dates were only 1.22 mg/kg, 0.68 mg/kg and 5.78 mg/kg for day-91 forage and beans and day-105 hay, respectively. Especially for forage these values are considerably lower than the residues arising from the fluorophenyl label, even after correction for the different application rates.

**Table 7.2.1.1-4 [Table 6.1.3b in DAR]. Distribution of metabolites (% of recovered radioactivity) in different crop parts after preemergence application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 to a sandy loam (1380 g a.i./ha)**

	Crop part (sampling date)					
	Forage (21 d)	Forage (48 d)	Beans (48 d)	Forage (91 d)	Beans (91 d)	Hay (105 d)
TRR = mg/kg	2.60	1.23	0.21 ❶	1.22	0.68	5.78
a.i.	n.d.	n.d.	-	n.d.	n.d.	n.d.
M 25	68	61	-	58	-	66
other Th-Conj.	-	-	-	19	-	13
M 34	-	-	-	-	66	-
Remainder ❷	-	-	-	-	27	-
Unknown	26	18	-	13	-	10
Not extract.	9	19	-	3	<1	5
Total ❸	103	98	-	93	93	94

❶: not further analysed due to insufficient amount of bean sample

❷: fractions not further analysed due to low amounts of radioactivity

❸: total does not equal 100 % due to rounding

M 25: N-glucoside conjugate of thiadone, THNG

M 34: N-malonylalanine conjugate of thiadone, THNMALALA

With the exception of the early forage samples, where no acid or base hydrolysis was performed, > 95 % of the TRR could be extracted.

In the 21-day forage sample the only major metabolite, accounting for 68 % of the TRR, was identified as the N-glucosyl conjugate of thiadone (ThN-glucoside, M 25). In the forage and hay sampled at days 48, 91 and 105 this metabolite accounted for 61 %, 58 % and 66 % of the recovered radioactivity, respectively. In addition, 4 other minor metabolites were characterised as thiadone conjugates, representing in total 13 % to 19 % of the TRR. Unknown radioactivity accounted for a maximum of 26 % of the recovered radioactivity, but no metabolite exceeded 9 % of the TRR. Due to insufficient sample material no further analyses were performed with the beans harvested on day 48. Only one single metabolite was observed in both the bean MeOH extract and MeOH reflux fractions of the day-91 sample. It accounted for 66 % of the recovered radioactivity and was tentatively identified as the N-malonylalanine conjugate of thiadone (M34). The parent compound was never detected in any of the samples.

### Comparative metabolism using <sup>19</sup>F NMR

The presence of fluorine in both rings of the FOE 5043 molecule afforded an opportunity to observe all of the metabolites by <sup>19</sup>F nmr. Using radiochromatographic techniques and <sup>19</sup>F nmr in their soybean metabolism study, Krolski & Bosnak (1995a) demonstrated that the nmr accurately reflected the metabolic fate of both the fluorophenyl and trifluoromethylthiadiazole portion of the molecule. In the next step they compared <sup>19</sup>F nmr spectra of the extracts from their above mentioned soybean study with samples taken from the studies on the metabolism of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 in corn and cotton mentioned above. It was confirmed by <sup>19</sup>F nmr that five metabolites resulting from the fluorophenyl portion of the molecule were present in all crops (M1, FOE oxalate; M2, FOE sulfonic acid; M4, FOE thioglycolate sulfoxide; M6, FOE methylsulfoxide; M7, FOE methylsulfone). A further metabolite (M33, FOE sulfinyl lactic acid) was observed in corn forage and fodder.

*In addition, the comparison of  $^{19}\text{F}$  nmr spectra of extracts from the different [fluorophenyl-UL- $^{14}\text{C}$ ]FOE 5043 treated crops with those from the [thiadiazole-2- $^{14}\text{C}$ ]FOE 5043 study in soybeans also revealed a comparable picture. Whereas in the cotton extract an additional minor metabolite containing the trifluoromethyl moiety and accounting for < 10 % of the total residue was observed, there was only a single trifluoromethyl resonance in the corn forage extract, which was attributed to M25 (ThN-glucoside).*

## Conclusion

**For the purpose of renewal of a.s. approval, this study was considered as acceptable.**

## Plant cell suspension cultures

<b>Report:</b>	Köster, J., Brauner, A., Degradation of [fluorophenyl-UL- $^{14}\text{C}$ ]FOE 5043 and [thiadiazole-2- $^{14}\text{C}$ ]FOE 5043 by heterotrophic plant cell suspension cultures, Bayer AC, report no.: PF4049 of 12.04.1995
<b>Guideline:</b>	no guideline applicable for this type of study
<b>GLP:</b>	yes (certified laboratory)

Description of this study evaluated by RMS (France) is cited below.

*The metabolism of [fluorophenyl-UL- $^{14}\text{C}$ ]- (specific radioactivity 66.6 mCi/mMole (radiochemical purity > 99 %) as well as [thiadiazole-2- $^{14}\text{C}$ ]FOE 5043 (specific radioactivity 18.47 mCi/mMole (radiochemical purity > 99 %) in heterotrophic cell suspension cultures of different plant species (soybean, wheat, peanut, corn and cotton) was investigated.*

*Although some differences between the species appeared a common metabolic pathway could be established. During the course of the experiments (usually 7 days) the parent compound was almost completely degraded. The degradation is initiated by cleavage of the molecule into the fluorophenylacetamide portion (A) and thiadiazole portion (B). These primary metabolites were directly conjugated with glutathione (GSH) or homogluthathione (hGSH)(A) and glucose (B). Whereas the stable N-glucoside of thiadone (M25) was the major metabolite resulting from this portion of the molecule, the glutathione/homogluthathione conjugates were further metabolised very rapidly to the FOE cysteine conjugate (M23). The cysteine conjugate is the central compound in the further metabolism of this part of the molecule and was the most important metabolite in the extracts of all cell cultures.*

*The results of the plant cell culture studies are in good agreement with those from metabolism studies with intact plants. The fact that the early intermediates (GSH/hGSH-conjugates and FOE cysteine conjugate) were only detected in plant cell cultures and that on the other hand some of the metabolites from intact plants were not detected may be attributed to the very short duration of the plant cell culture studies.*

## Conclusion

**For the purpose of renewal of a.s. approval, this study was considered as supportive information.**

### B 7.2.1.2 Additional plant metabolism studies with [fluorophenyl-UL- $^{14}\text{C}$ ]flufenacet

The following additional plant metabolism studies were conducted on potato, wheat and corn for registration in USA applying a higher application rate than used in Europe. These studies were not included in the original dossier submitted for Annex I inclusion. However, in the meantime they supported also registrations in these crops in European Member States at lower application rates and were evaluated on national level and for the review of existing MRLs according to Article 12 of Regulation (EC) 396/2005. They are now added to complete the picture on the metabolism of flufenacet in plants and to confirm common basic metabolic transformations.

**POTATO**

<b>Report:</b>	<b>KCA 6.2.1/07</b> , Beedle, E. C.; Ying, S. L.; 2000; M-020428-01_ <b>also filed KCA 4.1.2</b>
Title:	The Metabolism of [fluorophenyl-UL- <sup>14</sup> C]Flufenacet in Potatoes
Document No:	M-020428-01-1
Report No:	109226, dated 2000-04-28
Guidelines:	US-EPA OPPTS 860.1300, Nature of Residues - Plants
GLP	Yes; deviations: none

**Executive Summary**

The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in potatoes following two techniques of application: pre-emergent soil treatment at a use rate of 2.30 lb. ai/acre (2.58 kg as/ha) and post-emergent foliar treatment at a rate of 2.69 lb. ai/acre (3.01 kg as/ha). At harvest, mature tubers contained total radioactive residues (TRR) at a level of 0.35 mg equ/kg (soil treatment) or 0.32 mg equ/kg (foliar treatment). The tubers were homogenized under liquid nitrogen and extracted with methanol at room temperature and refluxed with methanol. The release of residues was completed by hydrolysis of the matrix with hydrochloric acid at room temperature. The extracted residues were separated by reversed phase HPLC and identified by LC-MS/MS and co-elution with authentic reference standards.

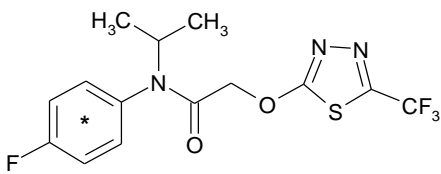
A total of 16 metabolites were detected in the tubers grown after soil treatment and a total of 13 metabolites in the tubers grown after foliar treatment. 63% of TRR was identified in the tubers after soil treatment and even 80% of TRR in the tubers after foliar treatment. Two major metabolites were identified in both trials. Most prominent was FOE cysteine (FACS, M23) amounting to 44% of TRR after soil treatment and to 52% of TRR after foliar treatment. The second major metabolite was identified as FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41) amounting to 19% of TRR after soil treatment and to 17% of TRR after foliar treatment. Two minor metabolites were tentatively identified in the tubers after foliar treatment, i.e. FOE thioglycolate sulfoxide (FAMSOC, M4) amounting to 7% of TRR and FOE sulfonic acid (FASO3H, M2) amounting to 4% of TRR. A lot of minor unknown metabolites were also detected in both trials, all of them at a level of < 10% of TRR. The non-extractable residues accounted for 4% of TRR in both trials. The parent substance flufenacet was not observed in the tubers of any treatment.

From the pattern of metabolites observed the initial step of flufenacet metabolism in potato tubers is assumed to be a glutathionate conjugation of the acetamide moiety of the molecule. The transient glutathionate degraded to FOE cysteine being the main residue component in potatoes. Subsequent metabolic steps are hydrolysis and oxidation of FOE cysteine followed by conjugation with glucose forming minor metabolites. The same metabolic pathway was also observed in soybean, corn and wheat, also conducted with [fluorophenyl-UL-<sup>14</sup>C]flufenacet. A metabolism study with [thiadiazole-5-<sup>14</sup>C] labeled flufenacet completed the metabolic pathway in potato (see below). The proposed metabolic pathway of flufenacet in potato tubers is shown in Figure 7.2.1.2-1.

The extraction of flufenacet residues according to the residue analytical method via oxidative hydrolysis and determination of the common moiety “4-fluorophenyl-*N*-isopropylamine” was complete when compared to the total amount of identified residue components in this metabolism study.

## Material and Methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluorophenyl)-<i>N</i>-isopropyl-2-((5-(trifluoromethyl)-[1,3,4]thiadiazol-2-yl)oxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[fluorophenyl-UL- <sup>14</sup> C]Flufenacet
Specific radioactivity	47.9 mCi/mmol (0.132 mCi/mg, 4.878 MBq/mg)
Radiochemical purity	>99% (radio-HPLC)

### Test Plants

Test plant	Potato ( <i>Solanum tuberosum</i> )
Variety	Kennebec
Growth stage at application	Two parallel trials: (1) Pre-emergent soil treatment at the same day as planting of the potatoes (2) Post-emergent foliar treatment approx. 4 weeks after emergence
Harvested commodities	Tubers, immature (only soil treatment) and mature

#### (1) Pre-emergent treatment:

##### Planting of seed potatoes, preparation and application of the test mixture

The radiolabelled test substance (dissolved in acetonitrile) was mixed the same amount of stable labelled [isopropyl-1,3-<sup>13</sup>C]flufenacet and with a blank formulation resulting in a 60WP formulation. The solvent was removed by rotary evaporation and water was added to yield the application mixture. An aliquot was taken for radio-HPLC analysis. The application rate corresponded to 2.30 lb. ai/acre (2.58 kg ai/ha).

Sandy loam (68.8% sand, 18.4% silt, 12.8% clay, 1.12% organic matter, pH 6.4) was filled in five 5-gal plastic buckets (approx. 19 L). Four seed potatoes were placed at a depth of 3 inches (7.5 cm) in each of the buckets. The upper 1 inch (2.5 cm) soil layer was removed and mixed with the application mixture in a tumbling mixer. The treated soil was returned to the buckets with the seed potatoes as top soil layer.

The potatoes were grown initially in a greenhouse. Following emergence (approx. 2 weeks after planting) they were thinned to one or two shoots per bucket. Further cultivation happened outdoors in a fenced patio in Stilwell, Kansas, USA, during spring and summer 1999 until harvest approx. 3.5 months after planting and soil treatment.

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## (2) Post-emergent treatment:

### Planting of seed potatoes, preparation and application of the test mixture

Four seed potatoes were planted in 5-gal plastic buckets with sandy soil as done for the pre-emergent treatment. The potatoes were first grown in a greenhouse, thinned after emergence and further cultivated under outdoor conditions in Stilwell, during spring and summer 1999 until harvest approx. 3.5 months after planting.

The radiolabelled test substance was mixed the  $^{13}\text{C}$ -labelled test substance, with WP60 formulation blank and with water as done for the pre-emergent treatment. The spray mixture was evenly sprayed to the leaf surface and the surrounding soil approx. 4 weeks after emergence using a hand-held plastic pump sprayer. An aliquot of the spray mixture was analysed by radio-HPLC. The application rate corresponded to 2.69 lb. ai/acre (3.01 kg ai/ha).

### Harvest and processing

Mature potatoes (109 day after pre-emergent and 67 days after post-emergent treatment) were dug out from the soil. The vines were cut away. The tubers were gently rinsed with water to remove soil, combined from all buckets of the same treatment type, cut into pieces and homogenized in liquid nitrogen using a high speed mixer. Following evaporation of the liquid nitrogen in a freezer the pulverized tubers were radioassayed. Aliquots of the tuber samples were used for initial extraction (6 days after harvest). The remaining samples were stored in a freezer at approx.  $-20^{\circ}\text{C}$ .

The homogenized tubers were extracted three times with methanol at room temperature, followed by 4-hours refluxing with methanol and hydrolyzed with 1N hydrochloric acid at ambient temperature for 8 hours. The acid hydrolysate was adjusted to pH 6 and extracted with chloroform. All liquid phases were radioassayed. The final solids were first air-dried and radioassayed *via* combustion.

To examine for potential glucoside conjugates, a major radioactive residue component was isolated by preparative HPLC, evaporated to dryness and re-dissolved in a sodium phosphate buffer solution. This solution was incubated with  $\beta$ -glucosidase ( $37^{\circ}\text{C}$ , 24 hours), then concentrated to dryness, re-dissolved in acidic methanol (0.1% acetic acid)/water (4/1) and analyzed by radio-HPLC.

### Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed  $^{14}\text{CO}_2$  absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.0005 mg parent equivalents/kg (0.0005 mg equ/kg).

Radio-HPLC was conducted on a RP8 column (250 x 10 mm, 5  $\mu\text{m}$  particle size) operated with a gradient mixture of water and methanol (both containing 0.1% acetic acid). The HPLC system was equipped with a radiomonitor with a glass scintillator. The linearity of the radiomonitor response was examined by injection of various amounts of radioactivity. The limit of detection was derived from detector-response curve and the specific radioactivity of the test substance amounting to 0.002 – 0.007  $\mu\text{g}$  of the test substance.

LC-MS/MS analyses were performed with a combination of a TSQ mass spectrometer connected to a HPLC system with a RP8 column (250 x 4.6mm, 5  $\mu\text{m}$  particle size) and a radiomonitor. A gradient mixture of aqueous ammonium acetate or formic acid and methanol served as mobile phase. The MS system was operated in the negative ion electrospray ionization mode.

## **Findings**

### Total radioactive residues and their extractability in potato tubers

The total radioactive residues (TRR) amounted to 1.77 mg equ/kg in immature tubers 40 days after soil (pre-emergence) treatment. In mature tubers, TRR amounted to 0.35 mg equ/kg 109 days after soil

treatment at a rate of 2.58 kg as/ha and to 0.32 mg equ/kg 67 days after foliar treatment at a rate of 3.01 kg as/ha.

The extractable portions of TRR using the different techniques are shown in Table 7.2.1.2-1. Most the residues could already be released by conventional extraction with methanol at ambient temperature accounting for 76 – 79% of TRR. Refluxing with methanol and hydrolysis with hydrochloric acid at room temperature almost completed the release of residues leaving only a small portion of non-extractable residues (4% of TRR, 0.01 mg equ/kg).

#### Residues in potato tubers following pre- and post-emergence treatment with flufenacet

The composition of the radioactive residues in mature potato tubers following pre- and post-emergence soil and foliar treatment is presented in Table 7.2.1.2-2. A total of 16 components were extracted from the tubers after soil treatment and a total of 13 components after foliar treatment. The parent substance was not observed in the tuber either after soil or after foliar treatment.

Two metabolites revealed to be the main residue components, i.e. P2, FOE cysteine (FACS, M23) amounting to 44% of TRR (0.16 mg equ/kg) after soil and to 52% of TRR (0.17 mg equ/kg) after foliar treatment and P1, FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M23) amounting to 19% of TRR (0.07 mg equ/kg) after soil and to 17% of TRR (0.05 mg equ/kg) after foliar treatment. These metabolites were isolated by radio-HPLC and identified by LC-MS making use of additional <sup>13</sup>C-labelling. The glucoside conjugation of FOE sulfanyl lactic acid was confirmed by enzymatic splitting off of glucose with glucosidase.

After foliar treatment, two additional metabolites could be identified in the tubers, i.e. FOE thioglycolate-sulfoxide (FAMSOC, M4) amounting to 7% of TRR (0.02 mg equ/kg) and FOE-sulfonic acid (FASO3H, M2) amounting to 4% of TRR (0.01 mg equ/kg). These metabolites were tentatively identified by co-chromatography with authentic reference standards.

From these residue components the following metabolic transformation reactions were concluded: The primary transformation was a glutathione conjugation of the fluorophenyl-isopropyl-acetamide moiety of flufenacet followed by hydrolytic release of alanine and glutamic acid to form FOE cysteine. Subsequent metabolic reactions were hydrolysis forming transient FOE sulfanyl lactic acid and FOE thioglycolate sulfoxide and oxidation of the sulfur to FOE sulfonic acid. FOE sulfanyl lactic acid was finally conjugated as glucoside. The proposed metabolic pathways are presented in Figure 7.2.1.2-1.

#### Extraction efficiency of the residue analytical method<sup>1</sup>

The extraction efficiency of the analytical method (accountability of residue method) was examined using potato tubers with incurred residues from the pre-emergent and post-emergent application of radiolabelled flufenacet. TRR levels of tubers used for this test amounted to 0.37 or 0.34 mg equ/kg after pre- or post-emergent application. These levels were slightly higher (approx. 6%) than the initial levels, probably due to desiccation during freezer storage.

Following oxidation, hydrolysis and steam distillation of the residues in tubers from post-emergent application the distillate contained a radioactivity level of 0.28 mg equ/kg. 0.26 mg equ/kg partitioned into dichloromethane and 0.24 mg equ/kg was quantified as the derivatized analytical target *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (0.25 mg equ/kg, Table 7.2.1.2-2) this figure represented an extraction efficiency of 96%.

The distillate from tubers grown in pre-emergent treated soil contained 0.31 mg equ/kg, and 0.28 mg equ/kg partitioned into dichloromethane. 0.26 mg equ/kg was quantified as the derivatized analytical target *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol

<sup>1</sup> Gould, T. J., Lemke, V. J. (1995). An analytical method for the determination of FOE 5043 residues in plant matrices, report 106406 of Bayer Corp., Stilwell, KS, USA, Comp. No. M-041601-01-1; now replaced by the current version (2013) without derivatization and direct HPLC-MS/MS determination of the common moiety, Comp. No. M-448503-01-1.

determined in the metabolism experiment (0.23 mg equ/kg, Table 7.2.1.2-2) this figure represented an extraction efficiency of 113%.

Therefore, it is concluded that the extraction efficiency of the analytical method from potato tubers is excellent when compared with the amount of all identified residue components detected in this metabolism study.

#### Storage stability of flufenacet residues in potato tubers

The initial extraction of the tubers following both soil and foliar treatment was performed within 6 days after sample collection. Re-extraction was performed on frozen samples 169 days after harvest. The major metabolites in both trials, FOE cysteine (FACS) and FOE sulfanyl lactic acid glucoside (FAMSL-Glu), were found to be stable upon storage. Also, the minor metabolites in tubers following foliar treatment were stable upon storage. Therefore, the stability of flufenacet residues in potato tubers was shown for a storage period of approx. 6 months at approx. -20°C.

#### **Conclusion**

The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in potatoes following pre-emergent soil treatment at a use rate of 2.30 lb. ai/acre (2.58 kg as/ha) and following post-emergent foliar treatment at a rate of 2.69 lb. ai/acre (3.01 kg as/ha). At harvest, mature tubers contained total radioactive residues (TRR) at a level of 0.35 mg equ/kg (soil treatment) or 0.32 mg equ/kg (foliar treatment).

A total of 16 metabolites were detected in the tubers grown after soil treatment and a total of 13 metabolites in the tubers grown after foliar treatment. Two major metabolites were identified in both trials. Most prominent was FOE cysteine (FACS, M23) amounting to 44% of TRR after soil treatment and to 52% of TRR after foliar treatment. The second major metabolite was identified as FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41) amounting to 19% of TRR after soil treatment and to 17% of TRR after foliar treatment. Two minor metabolites were detected in the tubers after foliar treatment, i.e. FOE thioglycolate sulfoxide (FAMSOC, M4) amounting to 7% of TRR and FOE sulfonic acid (FASO3H, M2) amounting to 4% of TRR. The parent substance flufenacet was not observed in the tubers of any treatment.

From the pattern of metabolites observed the initial step of flufenacet metabolism in potato tubers is assumed to be a glutathionate conjugation of the acetamide moiety of the molecule. The transient glutathionate degraded to FOE cysteine being the main residue component in potatoes. Subsequent metabolic steps are hydrolysis and oxidation of FOE cysteine followed by conjugation with glucose forming minor metabolites. The same metabolic pathway was also observed in soybean<sup>2</sup>, corn<sup>3,4</sup> and wheat<sup>5</sup>. All of these metabolism studies were conducted with [fluorophenyl-UL-<sup>14</sup>C]flufenacet. A metabolism study with [thiadiazole-5-<sup>14</sup>C] labeled flufenacet completed the metabolic pathway in potato<sup>6</sup>. The proposed metabolic pathway of flufenacet in potato tubers is shown in Figure 7.2.1.2-1.

The extraction of flufenacet residues according to the residue analytical method via oxidative hydrolysis and determination of the common moiety “4-fluorophenyl-*N*-isopropyl-amine” was complete when compared to the total amount of identified residue components in this metabolism study.

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<sup>2</sup> Krolski, M. E. and Bosnak, L. L. (1995): The metabolism of FOE 5043 in soybeans, Bayer AG, Div. Report No. MR105187, Comp. No. M-002278-01-1

<sup>3</sup> Baird, J. H. (1994): The metabolism of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 in corn, Bayer AG, Div. Report No. MR105027, Comp. No. M-002270-01-1

<sup>4</sup> Krolski, M. E. and Bosnak, L. L. (1998): The metabolism of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 in corn after postemergent foliar application, Bayer AG, Div. Report No. 108497, Comp. No. M-005755-01

<sup>5</sup> Krolski, M. E. and Bosnak, L. L. (1997): The metabolism of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 in wheat after postemergent foliar spray application, Bayer AG Div Report 107399, Comp. No. M-002275-01-1

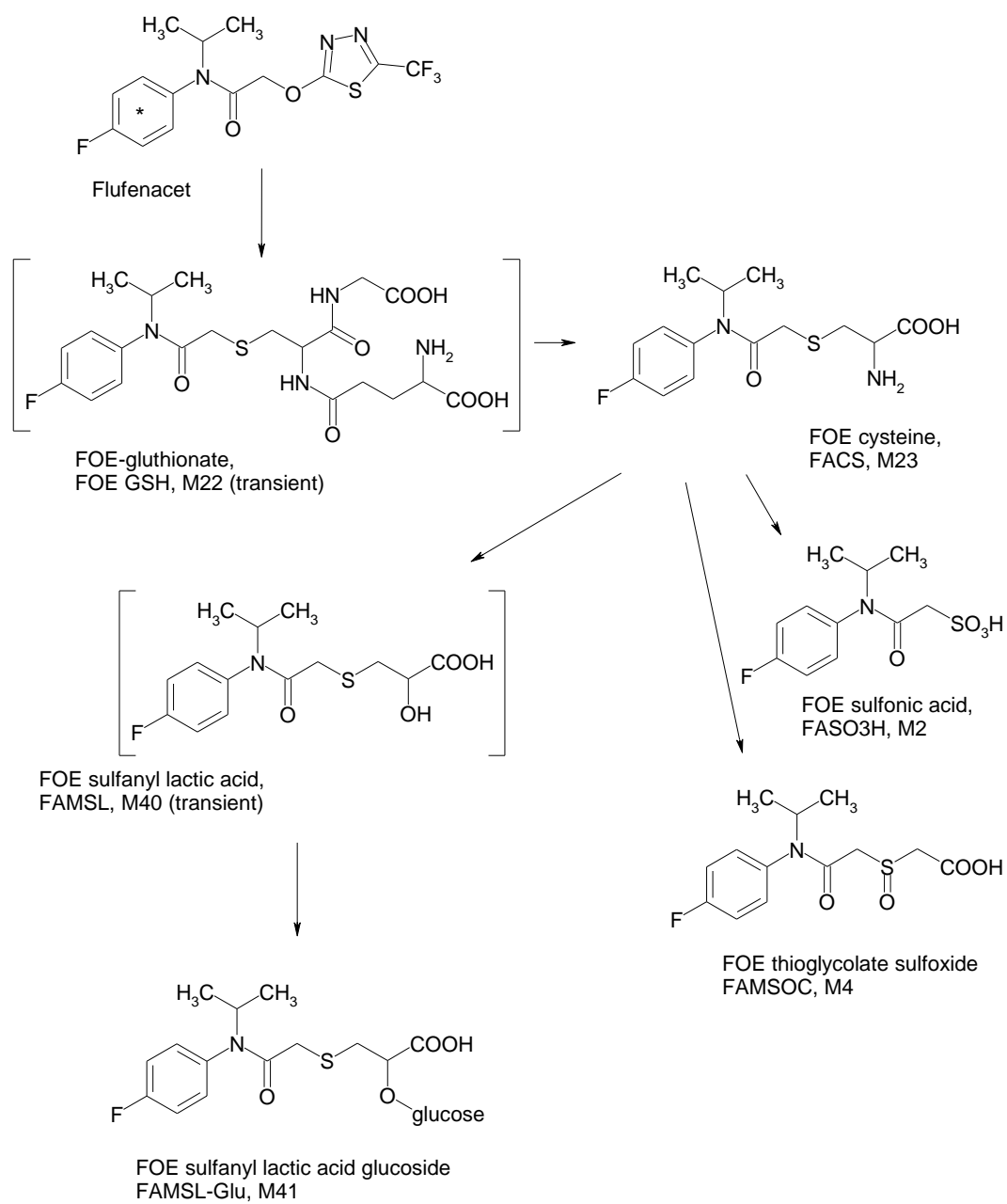
<sup>6</sup> Bongartz, R. (2012): Metabolism of [thiadiazole-5-<sup>14</sup>C]flufenacet in potatoes, report EnSa-12-0537 of Bayer CropScience, Comp. No. M-441506-02-1

**Table 7.2.1.2-1: Extractability of radioactive residues from mature potato tubers treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet**

Treatment type	Soil treatment, pre-emergent		Foliar treatment, post-emergent	
Application rate [kg as/ha]	2.58		3.01	
Days after treatment	109		67	
TRR [mg equ/kg]	0.35		0.32	
<b>Extraction with</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
Methanol, room temperature	79	0.28	76	0.24
Methanol, refluxing	7	0.02	8	0.03
1N HCl, room temperature	10	0.04	12	0.04
- Partition into chloroform	< 1	< 0.01	-	-
- Partition into water	10	0.04	-	-
<b>Non extractable (solids)</b>	4	0.01	4	0.01
<b>Total</b>	100	0.35	100	0.32

**Table 7.2.1.2-2: Composition of residues in mature potato tubers treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet (sum of the respective components in all extraction fractions)**

Treatment type	Soil treatment, pre-emergent		Foliar treatment, post-emergent	
Application rate [kg as/ha]	2.58		3.01	
Days after treatment	109		67	
TRR [mg equ/kg]	0.35		0.32	
<b>Metabolites</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
P1, FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41)	19	0.07	17	0.05
P2, FOE cysteine (FACS, M23)	44	0.16	52	0.17
P3 – P16, unknown	< 1 - 6	< 0.01 – 0.02	-	-
P17, FOE sulfonic acid (FASO3H, M2)	-	-	4	0.01
P19, FOE thioglycolate sulfoxide (FAMSOC, M4)	-	-	7	0.02
P18, P20 – P27, unknown	-	-	< 1 - 3	< 0.01 – 0.01
<b>Total identified</b>	63	0.23	80	0.25

**Figure 7.2.1.2-1: Proposed metabolic pathway of flufenacet in potato tubers after soil and foliar application**

\*  $^{14}\text{C}$  radiolabel      [ . ] transient

**WHEAT**

<b>Report:</b>	<b>KCA 6.2.1/05</b> , Krolski, M. E.; Bosnak, L. L.; 1997; M-002275-01_ <b>also filed KCA 4.1.2</b>
Title:	The Metabolism of [Fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in Wheat After Postemergent Foliar Application
Document No:	M-002275-01-1
Report No:	107399, dated 1997-11-04
Guidelines:	US-EPA OPPTS 860.1300, Nature of Residues - Plants
GLP	yes

**Executive Summary**

The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in spring wheat following post-emergent foliar application to young shoots (4-tiller growth stage) at a use rate of 0.46 lb. ai/acre (0.52 kg as/ha). Agricultural commodities of wheat were collected as immature forage, immature hay, mature straw and grain. All commodity samples were homogenized under liquid nitrogen and aliquots were radioassayed by combustion and liquid scintillation counting (LSC). The total radioactive residues (TRR) amounted to 1.93; 3.50; 2.04 and 0.62 mg equ/kg in forage, hay, straw and grain. Extraction with methanol at ambient temperature and under reflux revealed a high extractability of the radioactive residues accounting for 92, 94, 86 and 80% of TRR for forage, hay, straw and grain. Following further acid and alkaline hydrolysis of the residues non-extractable from plant matrix were negligible ( $\leq 3 - 4\%$  of TRR). The extracted residues were separated by reversed phase HPLC and identified by LC-MS/MS and co-elution with authentic reference standards.

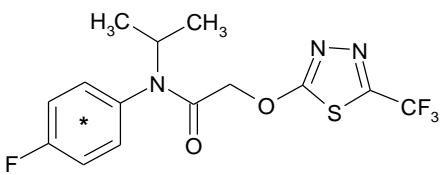
The metabolism of flufenacet in wheat was extensive. While no parent substance was observed in any of the plant commodities 12 metabolites were detected in forage and straw, and 9 metabolites in hay and grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in all commodities. It proved to be predominant in wheat grain amounting to 65% of TRR (corresponding to 0.40 mg equ/kg). Other metabolites in grain appeared at a very low level ( $\leq 2\%$  of TRR). In forage, hay and straw two other major metabolites were identified as FOE sulfinyl lactic acid I (FAMSOL I, M33) and FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41). In straw, a further metabolite FOE sulfonic acid (FOESO3H, M2) amounted to 15% of TRR.

The main metabolite present in all commodities, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. From the pattern of metabolites observed in this study with [fluorophenyl-UL-<sup>14</sup>C] labeled flufenacet a metabolic pathway of flufenacet in wheat is proposed in Figure 7.2.1.2-2. The parent substance was not observed in any commodity of forage, hay, straw and grain. All major metabolites in these commodities contained the common moiety fluorophenyl-*N*-isopropyl amine.

Comparative extraction of the residues using methanol (this metabolism study) and determination of the residues using the residue analytical method (oxidative acid hydrolysis and quantification of the hereby formed *N*-fluorophenyl-*N*-isopropyl amine) showed a good agreement of amount of residue compounds containing the common moiety.

## Material and Methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluorophenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[fluorophenyl-UL- <sup>14</sup> C]Flufenacet
Specific radioactivity	47.9 mCi/mmol (0.132 mCi/mg, 4.878 MBq/mg)
Radiochemical purity	96% (radio-HPLC), 92% after formulation with slight degradation to FOE alcohol (FOEALC, M3, identified by HPLC-MS)

### Test Plants

Test plant	Spring wheat ( <i>Triticum vulgare</i> )
Origin	Farmers Union Cooperative, Spring Hill, Kansas, USA
Growth stage at application	4-tiller growth stage, 46 days after seed planting
Harvested commodities	Forage, hay, straw, grain

#### Planting of wheat grain, preparation and application of the test mixture

Loam soil (49.2% sand, 32.8% silt, 18.0% clay, 2.51% organic matter, pH 6.4) was filled in a trough with a surface area of 18.4 ft<sup>2</sup> (1.70 m<sup>2</sup>) and a depth of 14 inches (35 cm). Wheat seeds were placed in furrows on the soil surface, approx. 6 inches (15 cm) apart, at approx. 1 cm intervals. The furrows were finally covered with a 0.5 cm soil layer. The wheat was grown outdoors in spring and summer 1995 at the Bayer Research Park in Stilwell, Kansas, USA.

The radiolabelled test substance was mixed 60WP blank formulation and water resulting in the spraying mixture. This spraying mixture was evenly sprayed across the surface of the trough with the wheat plants in the 4-tillering stage (46 days after sowing) using a plastic pump sprayer. The actual application rate corresponded to 0.461 lb. ai/acre (0.52 kg as/ha).

#### Harvest, processing and extraction

The wheat plants were harvested at the following growth stages:

Forage: at BBCH 26, 6-tillering growth stage, 64 days after sowing  
 Hay: at BBCH 85, soft dough growth stage  
 Straw and grain: at full maturity, 105 and 112 days after sowing

Plants were cut off at the soil surface level. They were cut into 1-inch pieces and homogenized under liquid nitrogen using a high-speed tissue mixer. The liquid nitrogen was allowed to evaporate in a freezer at < -10°C. Aliquots of the resulting tissue powder were radioassayed and the remainder stored in the freezer for later analysis.

In case of grain and straw sampling, ripe heads were first cut from the stalks using scissors. Then, the remaining plant (straw) was cut above the soil. The wheat heads were rubbed across a No. 10 soil sieve to remove the seeds. The sifted and winnowed (using a gentle nitrogen stream) grain was pulverized in a Warring blender. The straw was cut into pieces and homogenized under liquid nitrogen as done with forage and hay.

Homogenized forage was extracted with methanol (3x) at ambient temperature followed by refluxing with methanol. Aliquots of the methanol extracts were evaporated to dryness, re-dissolved in 0.1% acetic acid and analyzed by radio-HPLC. Each fraction was radioassayed.

Homogenized hay was extracted with methanol/water (3/1, 1x) and pure methanol (3x) at room temperature followed by refluxing with methanol. The methanol extracts were concentrated, and analyzed by radio-HPLC. The remaining solids were suspended successively in 1N hydrochloric acid and in 2N aqueous sodium hydroxide, both at ambient temperature. The aqueous phases were neutralized and partitioned against chloroform. The remaining solids were refluxed successively with 6N aqueous hydrochloric acid and 6N aqueous sodium hydroxide. All fractions/phases were radioassayed.

Homogenized straw and grain were extracted separately with methanol/water (4/1, 1x) following steeping at room temperature for half an hour. Extraction was continued with pure methanol (2x) at ambient temperature and under reflux, with hydrochloric acid and sodium hydroxide at room temperature and under reflux as done with hay. The aqueous phases were neutralized and partitioned against chloroform. Between acid/basic hydrolysis at room temperature and under reflux an additional extraction step with methanol/water (3/1) under ultrasonication was inserted. All fractions/phases were radioassayed.

#### Extraction efficiency of the residue analytical method<sup>7</sup>

Samples of grain and straw were processed and analyzed according to the analytical residue method for flufenacet in plants; this is a common moiety method with analysis for split-off “*N*-fluorophenyl-*N*-isopropyl amine”.

The sample was hydrolyzed and oxidized with sulfuric acid and potassium permanganate. Surplus permanganate was reduced by added sodium bisulfite. The hydrolysis was completed by addition of concentrated sulfuric acid and refluxing for 24 hours. The resulting mixture was cooled down, made strongly basic with sodium hydroxide and the formed *N*-fluorophenyl-*N*-isopropyl amine distilled off together with water (steam distillation). This amine was purified by partitioning with methylene chloride, derivatized with trifluoroacetic anhydride in pyridine. The final reaction mixture was radioassayed and analyzed by HPLC.

#### Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed <sup>14</sup>CO<sub>2</sub> absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.00077 mg parent equivalents/kg (0.00077 mg equ/kg) for liquid samples and 0.0011 mg equ/kg for solid samples.

Radio-HPLC was conducted on a RP8 or RP18 column (250 x 10 mm, 5 µm particle size) operated with a gradient mixture of water and methanol (both containing 0.1% acetic acid). The HPLC system was equipped with a radiomonitor with a glass scintillator. The linearity of the radiomonitor response was examined by injection of various amounts of radioactivity. The limit of detection was derived from

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<sup>7</sup> Gould, T. J., Lemke, V. J. (1995). An analytical method for the determination of FOE 5043 residues in plant matrices, report 106406 of Bayer Corp., Stilwell, KS, USA, Comp. No. M-041601-01-1; now replaced by the current version (2013) without derivatization and direct HPLC-MS/MS determination of the common moiety, Comp. No. M-448503-01-1.

detector-response curve and the specific radioactivity of the test substance. It amounted to 0.0093 µg of the test substance.

Radio-TLC of the straw hydrolysis fraction was conducted on TLC plates (5 x 20 cm) coated with Silicagel 60 F<sub>254</sub>. The plates were developed with tetrahydrofuran/methanol (9/1). Radioactive zones were detected using a radio-TLC-scanner.

LC-MS/MS analyses were performed with a combination of a mass spectrometer connected to a HPLC system. The MS system was operated in both the positive and negative ion electrospray ionization (ESI) mode.

## Findings

### Total radioactive residues and their extractability in wheat commodities

The total radioactive residues (TRR) amounted to 1.93 mg equ/kg in wheat forage 18 days post treatment, to 3.50 mg equ/kg in wheat hay 33 days post treatment, to 2.04 mg equ/kg in wheat straw 66 days post treatment and to 0.62 mg equ/kg in grain 59 - 66 days post treatment.

The extractable portions of TRR using the different techniques are shown in Table 7.2.1.2-3 for wheat forage and hay and in Table 7.2.1.2-4 for wheat straw and grain. Most the residues could already be released by conventional extraction with methanol at ambient temperature accounting for 64 (grain) - 92% (forage) of TRR. Refluxing with methanol released additional 4 - 16% of TRR resulting in a total of 80 (grain) – 96% (forage) of TRR. Sonication with methanol/water released an additional portion of 8% of TRR from wheat grain. Since most of the residues had already been released by the previous extraction steps succeeding acid and basic hydrolysis were not efficient. The portion of non-extractable residues finally was negligible amounting to 4% of TRR in forage (no acid or basic hydrolysis of the matrix performed), to <1% of TRR in hay, to 3% of TRR in straw and to 2% of TRR in grain samples.

### Residues in wheat commodities originating from foliar application of <sup>14</sup>C-flufenacet

The composition of the radioactive residues in wheat forage and hay following foliar treatment of [fluorophenyl-UL-<sup>14</sup>C]flufenacet are summarized in Table 7.2.1.2-5. The respective composition of residues in wheat straw and grain is shown in Table 7.2.1.2-6. A total of 12 metabolites were detected in forage and straw and 9 metabolites in hay and grain. The metabolites were identified by comparison of their HPLC retention to those of authentic reference standards and by individual collection following HPLC separation and identification by HPLC-MS.

The chromatographic profiles of the methanol extracts of forage, hay and straw were very similar. Common major metabolites were identified as FOE oxalate, M1 (14 – 36% of TRR) and FOE sulfinyl lactic acid I, M33 (20 – 26% of TRR). At the earlier growth stages forage and hay two additional metabolites were observed at relevant amounts, i.e. FOE sulfanyl lactic acid glucoside, M41 (8 – 21% of TRR) and FOE sulfinyl lactic acid glucoside, M37 (6 – 10% of TRR), whereas at maturity FOE sulfonic acid, M2 (15% of TRR) was found in straw. Other metabolites appeared at a minor extent (<10% of TRR).

The grain extract comprised mainly of a single component (65% of TRR corresponding to 0.40 mg equ/kg) which was identified as FOE oxalate, M1. Other metabolites were quantified as very minor (≤ 2% of TRR).

The parent substance was not observed in any commodity of forage, hay, straw and grain. All major metabolites in these commodities contained the common moiety “fluorophenyl-*N*-isopropyl amine”. The proposed metabolic pathway of flufenacet in wheat is shown in Figure 7.2.1.2-2.

### Extraction efficiency of the residue analytical method

The extraction efficiency of the analytical method (accountability of residue method) was examined using grain and straw with incurred residues from the current wheat metabolism study. TRR levels of grain and straw samples used for this test amounted to 0.55 and 1.96 mg equ/kg. These levels were slightly lower than the initial levels, probably due to hydration of the dried grain and straw during freezer storage.

Following oxidation, hydrolysis and steam distillation of formed common moiety *N*-fluorophenyl-*N*-isopropyl amine from wheat grain the distillate contained 97% of TRR in the original grain sample. 84% of TRR partitioned into the organic phase after addition of sodium hydroxide. Subsequent derivatisation revealed the analytical target *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide representing 81% of TRR in the original grain sample. Compared to the total extractability with methanol determined in the metabolism experiment (80% of TRR extractable at room temperature and under reflux conditions, with 66% of TRR identified as metabolites containing the common moiety, Table 7.2.1.2-4) this figure represented a complete extraction of those residue components that contain the respective *N*-fluorophenyl-*N*-isopropyl amine moiety.

Applying the same method to a straw sample resulted in 86% of TRR in the distillate with 76% of TRR in the organic phase prior to derivatisation. The derivatized sample contained 70% of TRR in the original straw sample, which was identified as *N*-4-fluorophenyl-*N*-isopropyl-trifluoroacetamide. Compared to the total extractability with methanol determined in the metabolism experiment (86% of TRR extractable at room temperature and under reflux conditions, with 74% of TRR identified as metabolites containing the common moiety, Table 7.2.1.2-4) this figure represented also a complete extraction of those residue components that contain the respective *N*-fluorophenyl-*N*-isopropyl amine moiety.

#### Storage stability of residues in the freezer

Initial extraction of all commodities was made one month after sample collection. All extractions and quantitative measurements were completed within 6 months of sample collection. Therefore, no additional storage stability data are required according to OECD Guideline 501 (2007) on “Metabolism in Crops” to support this study.

### **Conclusion**

The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in spring wheat following post-emergent foliar application to young shoots (4-tiller growth stage) at an use rate of 0.46 lb. ai/acre (0.52 kg as/ha). The following crop commodities were collected and analysed: immature forage, immature hay, mature straw and grain. The total radioactive residues (TRR) amounted to 1.93; 3.50; 2.04 and 0.62 mg equ/kg in forage, hay, straw and grain. Extraction with methanol at ambient temperature and under reflux revealed a high extractability of the radioactive residues accounting for 92, 94, 86 and 80% of TRR for forage, hay, straw and grain. Following additional acid and alkaline hydrolysis of the plant matrix the non-extractable residues were negligible ( $\leq 3 - 4\%$  of TRR).

The metabolism of flufenacet was extensive in wheat. While no parent substance was observed in any of the plant commodities 12 metabolites were detected in forage and straw, and 9 metabolites in hay and grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in all commodities. It proved to be predominant in wheat grain amounting to 65% of TRR (corresponding to 0.40 mg equ/kg). Other metabolites in grain appeared at a very low level ( $\leq 2\%$  of TRR). In forage, hay and straw two other major metabolites were identified as FOE sulfinyl lactic acid I (FAMSOL I, M33) and FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41). In straw, a further metabolite FOE sulfonic acid (FOESO3H, M2) amounted to 15% of TRR.

The main metabolite present in all commodities, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. A similar metabolic pathway of flufenacet was also found in soybeans, corn and cotton<sup>8</sup>. All of these metabolism studies were conducted with [fluorophenyl-UL-<sup>14</sup>C]flufenacet. From the pattern of detected metabolites a metabolic pathway of flufenacet in wheat is proposed in Figure 7.2.1.2-2. A metabolism study with [thiadiazole-5-<sup>14</sup>C] labelled flufenacet completed the metabolic pathway in wheat<sup>9</sup> (see below).

<sup>8</sup> Krolski, M. E. and Bosnak, L. L. (1995): The metabolism of FOE 5043 in cotton, Bayer AG Div. Report No. 106666, Comp. No. M-002277-01-1

<sup>9</sup> Bongartz, R. and Miebach, D. (2013): Metabolism of [thiadiazole-5-<sup>14</sup>C]flufenacet in wheat, unpublished report EnSa-12-0536 of Bayer CropScience AG, Comp. No. M-444475-01-1

Comparative extraction of the residues using methanol (this metabolism study) and determination of the residues using the residue analytical method (oxidative acid hydrolysis and quantification of the hereby formed N-fluorophenyl-N-isopropyl amine) showed a good agreement of amount of residue compounds containing the common moiety.

**Table 7.2.1.2-3: Extractability of radioactive residues from wheat forage and hay following foliar treatment with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 0.52 kg as/ha**

Agricultural commodity	Wheat forage		Wheat hay	
Days after treatment	18		33	
TRR [mg equ/kg]	1.93		3.50	
<b>Extraction with</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
Methanol, room temperature	92	1.78	88	3.01
Methanol, refluxing	4	0.08	6	0.21
1N HCl, room temperature				
- Partition into chloroform	-	-	<1	<0.01
- Partition into water	-	-	2	0.02
2 N NaOH, room temperature				
- Partition into chloroform	-	-	<1	<0.01
- Partition into water	-	-	<1	<0.01
Methanol/water sonication	-	-	<1	0.01
6 N HCl, reflux	-	-	<1	<0.01
6 N NaOH, reflux	-	-	<1	<0.01
<b>Non-extractable (solids)</b>	4	0.08	<1	<0.01
<b>Total*</b>	100	1.94	100	3.37

\* slight differences from TRR determination measured by combustion due to rounding of subfractions

**Table 7.2.1.2-4: Extractability of radioactive residues from wheat straw and grain following foliar treatment with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 0.52 kg as/ha**

Agricultural commodity	Wheat straw		Wheat grain	
Days after treatment	66		59 - 66	
TRR [mg equ/kg]	2.04		0.62	
<b>Extraction with</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
Methanol, room temperature	76	1.51	64	0.36
Methanol, refluxing	10	0.20	16	0.09
1N HCl, room temperature				
- Partition into chloroform	<1	<0.01	<1	<0.01
- Partition into water	3	0.06	3	0.02
2 N NaOH, room temperature				
- Partition into chloroform	1	0.02	1	<0.01
- Partition into water	4	0.08	3	0.02
Methanol/water sonication	2	0.04	8	0.04
6 N HCl, reflux				
- Partition into chloroform	<1	<0.01	2	<0.01
- Partition into water	<1	<0.01	1	<0.01
6 N NaOH, reflux	<1	<0.01	<1	<0.01
<b>Non-extractable (solids)</b>	3	0.06	2	0.01
<b>Total*</b>	100	1.97	100	0.54

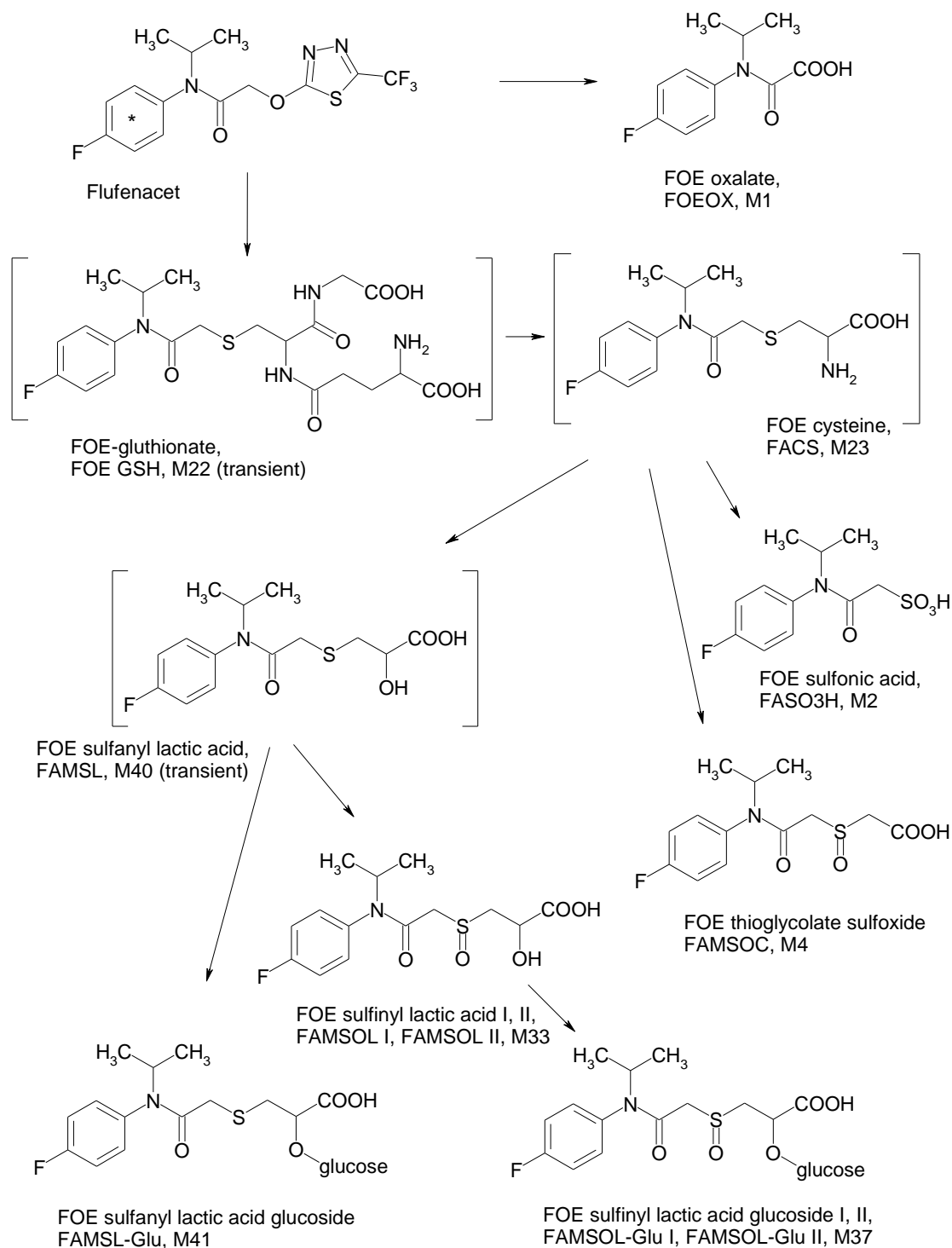
\* slight differences from TRR determination measured by combustion due to rounding of subfractions

**Table 7.2.1.2-5: Composition of residues in wheat forage and hay treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 0.52 kg as/ha**

<b>Agricultural commodity</b>	<b>Wheat forage</b>		<b>Wheat hay</b>	
Days after treatment	18		33	
TRR [mg equ/kg]	1.93		3.50	
<b>Metabolites</b> extracted with MeOH at RT and MeOH refluxing	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
Unknown 1	<1	<0.02	-	-
FOE oxalate (FOEOX, M1)	19	0.37	36	1.26
Unknown 2	-	-	<1	<0.04
FOE sulfinyl lactic acid glucoside I (FAMSOL-Glu I, M37)	6	0.12	10	35
FOE sulfinyl lactic acid glucoside II (FAMSOL-Glu II, M37)	6	0.12	5	0.18
FOE thioglycolate sulfoxide (FAMSOC, M4)	2	0.04	4	0.14
FOE sulfinyl lactic acid I (FAMSOL I, M33)	23	0.44	20	0.70
FOE sulfinyl lactic acid I I (FAMSOL II, M33)	7	0.14	4	0.14
Unknown 3	3	0.06	2	0.07
Unknown 4	2	0.04	-	-
FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41)	21	0.21	8	0.28
Unknown 5	<1	<0.02	-	-
Unknown 6	<1	<0.02	-	-
Total	89	1.74	89	3.12
<b>Total identified</b>	84	1.64	87	1.68

**Table 7.2.1.2-6: Composition of residues in wheat straw and grain treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 0.52 kg as/ha**

Agricultural commodity	Wheat straw		Wheat grain	
Days after treatment	66		59 - 66	
TRR [mg equ/kg]	2.04		0.62	
<b>Metabolites</b> extracted with MeOH at RT and MeOH refluxing	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	-	-	1	<0.01
FOE sulfonic acid (FASO3H, M2)	15	0.31	-	-
FOE oxalate (FOEOX, M1)	14	0.29	65	0.40
Unknown 2			2	0.01
FOE sulfinyl lactic acid glucoside I (FAMSOL-Glu I, M37)	2	0.04	<1	<0.01
FOE sulfinyl lactic acid glucoside II (FAMSOL-Glu II, M37)	1	0.02	1	<0.01
FOE thioglycolate sulfoxide (FAMSOC, M4)	7	0.14	-	-
Unknown 3	1	0.02	<1	<0.01
FOE sulfinyl lactic acid I (FAMSOL I, M33)	26	0.53	-	-
FOE sulfinyl lactic acid II (FAMSOL II, M33)	9	0.18	-	-
Unknown 4	1	0.02	<1	<0.01
Unknown 5	-	-	<1	<0.01
FOE sulfanyl lactic acid glucoside (FAMSL-Glu, M41)	<1	0.02	-	-
Unknown 6	<1	<0.02	<1	<0.01
Unknown 7	2	0.04	<1	<0.01
Total	78	1.61	69	0.41
<b>Total identified</b>	74	1.53	66	0.40

**Figure 7.2.1.2-2: Proposed metabolic pathway of flufenacet in wheat following post-emergent foliar application**

\* <sup>14</sup>C radiolabel [ . ] transient

## CORN

<b>Report:</b>	<b>KCA 6.2.1/06</b> , Krolski, M. E., Bosnak, L. L. (1998), M-005755-01-1
Title:	The Metabolism of [Fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in Corn After Postemergent Foliar Application
Document No:	M-005755-01-1
Report No:	108497, dated 1998-09-23
Guidelines:	US-EPA OPPTS 860.1300, Nature of Residues - Plants
GLP	Yes; deviation: none

## Executive Summary

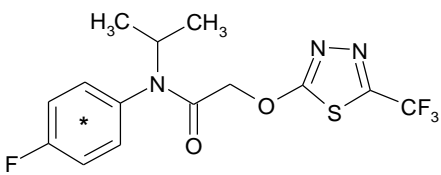
The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in corn/maize following post-emergent foliar application to young shoots (4 - 5 leaf growth stage) at an use rate of 1.30 lb. ai/acre (1.46 kg as/ha). Agricultural commodities of corn were collected as immature forage (82 day post treatment) and mature fodder and grain (129 days post treatment). All commodity samples were homogenized with dry ice and aliquots were radioassayed by combustion and liquid scintillation counting (LSC). The total radioactive residues (TRR) amounted to 0.62; 1.91 and 0.11 mg equ/kg in forage, fodder and grain. Extraction with methanol at ambient temperature released a very high portion of radioactive residues from animal feed commodities, i.e. forage accounting for 92% of TRR and fodder accounting for 82% of TRR. The extractability with methanol was lower from grain accounting for 47% of TRR at room temperature and additional 6% by refluxing. Relevant portions the residues in grain could be released by acidic hydrolysis of the matrix (11% by agitation with 1N HCl at room temperature and additional 14% with 6N HCl under reflux). These residues proved to be mainly polar. The extracted residues were separated by reversed phase HPLC and identified by LC-MS/MS and co-elution with authentic reference standards.

Flufenacet was extensively metabolized in corn. While no parent substance was observed in any of the plant commodities 7 metabolites were detected in forage, 10 metabolites in fodder and 6 metabolites in grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in animal feed commodities forage and fodder, but was absent in grain. The main metabolite in grain was identified as FOE sulfinyl lactic acid glucoside (FAMSOL-Glu, M37) amounting to 23% of TRR (0.02 mg equ/kg). This metabolite was also major in fodder (18% of TRR), but minor in forage (<10% of TRR). Exclusively in forage, the conjugate FOE malonylcysteine (FAMS-MalCys, M42) was observed at a significant extent (25% of TRR). A lot of other metabolites were detected in grain, fodder and forage, all of them containing the common moiety N-fluorophenyl-*N*-isopropyl amine.

The forage and fodder metabolite, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. From the pattern of metabolites observed in this study with [fluorophenyl-UL-<sup>14</sup>C] labelled flufenacet a metabolic pathway of flufenacet in corn is proposed in Figure 7.2.1.2-3.

## Material and Methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluorophenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[fluorophenyl-UL- <sup>14</sup> C]Flufenacet
Specific radioactivity	Originally: 47.9 mCi/mmol (0.132 mCi/mg, 4.878 MBq/mg), Used in the study: 146000 dpm/μg (2.433 MBq/mg, 0.066 mCi/mg)
Radiochemical purity	100% (radio-HPLC)

### Test Plants

Test plant	Corn ( <i>Zea mays</i> )
Variety	Great Lakes 533
Origin	Bayer Research Farm at Howe, Illinois, USA
Growth stage at application	4 – 5 leaf stage, 14 days after planting
Harvested commodities	Immature forage, fodder and grain at maturity

#### Planting of corn, preparation and application of the test mixture

Loam soil (49.2% sand, 32.8% silt, 18.0% clay, 2.51% organic matter, pH 6.4) was filled into twelve 5-gal (approx. 19 L) plastic buckets with drainage holes to a depth of 12 inches (approx. 30 cm). Several corn seeds were sown into each bucket. Following emergence the corn shoots were twice thinned to finally one plant per bucket. The corn plants were first grown outdoors in summer 1994 on a patio at the Bayer Research Park in Stilwell, Kansas, USA, and then matured in a greenhouse.

The radiolabelled test substance dissolved in ethyl acetate was blended with the same amount of non-labelled test substance and mixed with 60WP formulation blank. Following thorough mixing the solvent was evaporated and water was added resulting in the spray mixture. This mixture was sonicated and then transferred to a hand-held plastic pump sprayer. The young corn plants at the 4- to 5-leaf stage were evenly sprayed with the spray mixture 14 days after planting. The actual application rate was 1.30 lb. ai/acre (1.46 kg as/ha).

#### Harvest and processing

Agricultural commodities of corn plants were harvested at the two following growth stages:

Forage: at BBCH 85-86, 82 days post treatment at the late dough/early dent stage  
 Fodder and grain: at BBCH 97, 129 days post treatment at maturity

Immature plants were cut off few inches above the soil surface level. They were cut into 5-6-inch pieces and pulverized in a food processor in presence of dry ice. The dry ice was allowed to sublime off in a freezer (< -10°C).

In case of mature plants, the ears were first removed from the stalks and husks. Dry grains were manually removed from each cob, processed in a food processor with dry ice and subsequently pulverized more finally in a blender also with dry ice. The remaining husks and cobs were added to the stalks (representing the fodder) and homogenized in a food processor with dry ice. The dry ice was allowed to sublime at  $< -10^{\circ}\text{C}$ . Aliquots of all homogenized samples were radioassayed. The remainder was stored under frozen condition until extraction and analysis.

#### Extraction of forage

Homogenized forage was extracted with methanol (3x) at ambient temperature followed by refluxing with methanol. Aliquots of the methanol extracts were evaporated to dryness, re-dissolved in aqueous 0.1% acetic acid and analyzed by radio-HPLC. The solids remaining after extraction were suspended successively in 1N hydrochloric acid and in 2N aqueous sodium hydroxide, both at ambient temperature. The aqueous phases were neutralized and partitioned against chloroform. The filtered solids were suspended in methanol/water (3/1) and sonicated for 2 hours at room temperature. Each fraction was radioassayed.

#### Extraction of fodder

Homogenized fodder was first steeped in methanol/water (4/1) and the extracted with the same solvent mixture followed by two extractions with pure methanol. The combined extract was concentrated and analyzed by radio-HPLC. The remaining solid was refluxed with methanol and the organic extract concentrated and analyzed by radio-HPLC.

The remaining solids were suspended successively hydrolyzed with 1N hydrochloric acid and 2N sodium hydroxide followed by sonication in methanol/water (3/1) as mentioned above. The remaining solids were refluxed successively with 6N aqueous hydrochloric acid and 6N aqueous sodium hydroxide. All fractions/phases were radioassayed.

#### Extraction of grain

Homogenized grain powder was first steeped in methanol/water (4/1) and the extracted with the same solvent mixture followed by two extractions with pure methanol as conducted with fodder. The combined extract was concentrated and analyzed by radio-HPLC. The remaining solid was refluxed with methanol and the organic extract concentrated and analyzed by radio-HPLC.

The remaining solids were extracted successively with hexane (1x) and acetonitrile (2x). Then, the solids were refluxed with methanol and hydrolyzed with 1N hydrochloric acid and 2N aqueous sodium hydroxide at ambient temperature. The aqueous phase of the acid hydrolysis was partitioned against chloroform. The remaining solids were then sonicated in methanol/water (3/1) and finally hydrolyzed with 6N hydrochloric acid and 6N sodium hydroxide under reflux. The aqueous hydrolyzates were partitioned against chloroform. All fractions/phases were radioassayed.

#### Radioassaying and analysis

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed  $^{14}\text{CO}_2$  absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.00031 mg parent equivalents/kg (0.00031 mg equ/kg) for liquid samples and 0.00042 mg equ/kg for solid samples.

Radio-HPLC was conducted on a RP8 or RP18 column (250 x 10 mm, 5 or 10  $\mu\text{m}$  particle size) operated with a gradient mixture of water and methanol (both containing 0.1% acetic acid). The HPLC system was equipped with UV detector and a radiomonitor with a glass scintillator. The linearity of the radiomonitor response was examined by injection of various amounts of radioactivity. The limit of detection was derived from detector-response curve and the specific radioactivity of the test substance amounting to 0.0188  $\mu\text{g}$  of the test substance. In addition, a straight phase HPLC system was used for purification of isolated

metabolites operating with a normal phase column (250 x 10 mm, 5µm particle size) and a gradient of the solvents hexane and 0.2% acetic acid in IPA (isopropyl alcohol).

Radio-TLC of the fodder isolated metabolites was conducted on TLC plates (5 x 20 cm) coated with Silicagel 60 F<sub>254</sub>. The plates were developed with tetrahydrofuran/methanol (9/1). Radioactive zones were detected using a radio-TLC-scanner.

LC-MS/MS analyses were performed with a combination of a mass spectrometer connected to a HPLC system. The MS system was operated in both the positive and negative ion electrospray ionization (ESI) mode.

## Findings

### Total radioactive residues and their extractability in corn commodities

The total radioactive residues (TRR) amounted to 0.62 mg equ/kg in corn forage 82 days post treatment, to 1.91 mg equ/kg in fodder and to 0.11 mg equ/kg in grain both harvested 129 days post treatment.

Residues extractable from corn forage and fodder are shown in Table 7.2.1.2-7. Most the residues in forage and fodder could already be released by conventional extraction with methanol at ambient temperature accounting for 92% of TRR in forage and 82% of TRR in fodder. Refluxing with methanol released additional 2 - 6% of TRR resulting in a total of 94% (forage) and 88% (fodder) of TRR. Minor portions of residues were additionally released by acid and alkaline hydrolysis of the matrix and sonication with methanol and water. The non-extractable residues at the end of the extraction steps amounted to 1% of TRR

The extractable portion of TRR from corn grain is shown in Table 7.2.1.2-8. Extraction with methanol released only 47% of TRR at room temperature and additional 6% by refluxing. Relevant portions of the radioactive residues could also be released by mild acidic hydrolysis of the matrix at room temperature (2% of TRR being unpolar and 9% being polar) and drastic acidic hydrolysis with 6N HCl under reflux (3% of TRR unpolar and 14% polar). Minor portions were released by alkaline hydrolysis. The non-extractable residues amounted to 5% of TRR.

### Residues in corn commodities originating from foliar application of <sup>14</sup>C-flufenacet

The composition of the radioactive residues in corn forage and fodder following foliar treatment of [fluorophenyl-UL-<sup>14</sup>C]flufenacet is summarized in Table 7.2.1.2-9. The respective composition of residues in corn grain is shown in Table 7.2.1.2-10. A total of 7 metabolites were detected in forage, a total of 10 metabolites in corn fodder, and 6 metabolites in grain. The metabolites were identified by comparison of their HPLC characteristics to authentic reference standards and already identified metabolites from other plant commodities, and by individual collection following HPLC separation and identification by HPLC-MS.

The chromatographic profiles of the methanol extracts of forage, and fodder were similar. Common major metabolites were identified as FOE oxalate and M1 (22 - 27% of TRR) and FOE sulfinyl lactic acid I, M33 (16 - 19% of TRR). In corn forage, FOE sulfanyl lactic acid glucoside, M41 (25% of TRR) was observed as additional major metabolite, whereas FOE malonylcysteine, M42 (16% of TRR) was the analogue major metabolite in fodder. Corn fodder contained also FOE sulfinyl lactic acid glucoside, M37 (18% of TRR) as a major metabolite.

The grain extract comprised mainly of a single component (23% of TRR corresponding to 0.02 mg equ/kg) which was identified as FOE sulfinyl lactic acid glucoside, M37 (two diastereomers, non-separated). Apart from FOE thioglycolate sulfoxide, M4 (9% of TRR) and FOE methyl sulfoxide, M6 (7% of TRR), other metabolites were quantified as minor (≤ 4% of TRR).

The parent substance was not observed in any commodity forage, fodder and grain. All of the major metabolites in these commodities contained the common moiety “*N*-fluorophenyl-*N*-isopropyl amine”. The proposed metabolic pathway of flufenacet in corn is shown in Figure 7.2.1.2-3.

#### Storage stability of residues in the freezer

Initial extraction and analyses of plant samples were performed within 10 days after sample collection. Some samples were stored frozen for up to 13 months to repeat analysis. In addition, the storage stability of flufenacet residues at  $-26\pm5^{\circ}\text{C}$  was shown in a separate report using corn, soybean and turnip with incurred residues for at least 20 or 28 months<sup>10</sup>.

#### **Conclusion**

The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in corn following post-emergent foliar application to young shoots (4-5 leaf growth stage) at an use rate of 1.30 lb. ai/acre (1.46 kg as/ha). The following crop commodities were collected and analysed: immature forage, mature fodder (stalks, husk and cobs) and grain. The total radioactive residues (TRR) amounted to 0.62; 1.91 and 0.11 mg equ/kg in forage, fodder and grain. Extraction with methanol at ambient temperature revealed a high extractability of the radioactive residues from forage and fodder accounting for 92% of TRR (forage) and 82% of TRR (fodder). The extractability with methanol from grain was lower accounting for 47% of TRR at room temperature and additional 6% by refluxing. Relevant portions the residues in grain could be released by acidic hydrolysis of the matrix (11% by agitation with 1N HCl at room temperature and additional 14% with 6 N HCl under reflux). These residues proved to be mainly polar.

Flufenacet was extensively metabolized in corn. While no parent substance was observed in any of the plant commodities 7 metabolites were detected in forage, 10 metabolites in fodder and 6 metabolites in grain, respectively. FOE oxalate (FOEOX, M1) revealed to be a major metabolite in animal feed commodities forage and fodder, but was absent in grain. The main metabolite in grain was identified as FOE sulfinyl lactic acid glucoside (FAMSOL-Glu, M37) amounting to 23% of TRR (0.02 mg equ/kg). This metabolite was also major in fodder (18% of TRR), but minor in forage (<10% of TRR). Exclusively in forage, the conjugate FOE malonylcysteine (FAMS-MalCys, M42) was observed at a significant extent (25% of TRR). A lot of other metabolites were detected in grain, fodder and forage, all of them containing the common moiety “*N*-fluorophenyl-*N*-isopropyl amine”.

The forage and fodder metabolite, i.e. FOE oxalate, most likely arose from oxidation of transient primary alcohol hydrolysis product. All other metabolites were formed by hydrolysis, oxidation and conjugation of a primary transient metabolite formed by initial conjugation with glutathione. A similar metabolic pathway of flufenacet was also found in soybeans, wheat<sup>11</sup> and cotton.

From the pattern of metabolites observed in this study with [fluorophenyl-UL-<sup>14</sup>C] labelled flufenacet a metabolic pathway of flufenacet in wheat is proposed in Figure 7.2.1.2-3.

<sup>10</sup> Bosnak, L. L. (1995): The storage stability of FOE 5043 and metabolites in corn, soybean, and turnip raw agricultural commodities, unpublished report 106971 of Bayer Corp., Stilwell, Kansas, USA, Comp. No. M-002426-01-1.

<sup>11</sup> Krolski, M. E, and Bosnak, L. L. (1997): The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet in wheat after postemergent foliar application, Bayer AG, Div. Agriculture Report 107399, Comp. No. M-002275-01-1

**Table 7.2.1.2-7: Extractability of radioactive residues from corn forage and fodder following foliar treatment with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 1.46 kg as/ha**

Agricultural commodity	Corn forage		Corn fodder	
Days after treatment	82		129	
TRR [mg equ/kg]	0.62		1.91	
<b>Extraction with</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
Methanol, room temperature	92	0.54	82	1.53
Methanol, refluxing	2	0.01	6	0.11
1N HCl, room temperature				
- Partition into chloroform	<1	<0.01	<1	0.01
- Partition into water	1	<0.01	6	0.10
2 N NaOH, room temperature				
- Partition into chloroform	1	<0.01	<1	<0.01
- Partition into water	1	<0.01	2	0.04
Methanol/water sonication	<1	<0.01	2	0.04
6 N HCl, reflux	-	-	<1	<0.01
6 N NaOH, reflux	-	-	<1	<0.01
<b>Non-extractable (solids)</b>	1	<0.01	1	0.02
<b>Total*</b>	98	0.55	99	1.85

\* slight differences from TRR determination measured by combustion due to rounding of subfractions

**Table 7.2.1.2-8: Extractability of radioactive residues from grain of corn following foliar treatment with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 1.46 kg as/ha**

Agricultural commodity	Corn grain	
Days after treatment	129	
TRR [mg equ/kg]	0.11	
<b>Extraction with</b>	<b>[% of TRR]</b>	<b>[mg equ/kg]</b>
Methanol, room temperature	47	0.05
Hexane, room temperature	3	<0.01
Acetonitrile, room temperature	<1	<0.01
Methanol, refluxing	6	0.01
1N HCl, room temperature		
- Partition into chloroform	2	<0.01
- Partition into water	9	0.01
2 N NaOH, room temperature		
- Partition into chloroform	3	<0.01
- Partition into water	5	<0.01
Methanol/water sonication	2	
6 N HCl, reflux		
- Partition into chloroform	3	<0.01
- Partition into water	14	0.01
6 N NaOH, reflux		
- Partition into chloroform	<1	<0.01
- Partition into water	<1	<0.01
<b>Non-extractable (solids)</b>	5	<0.01
<b>Total*</b>	99	0.08

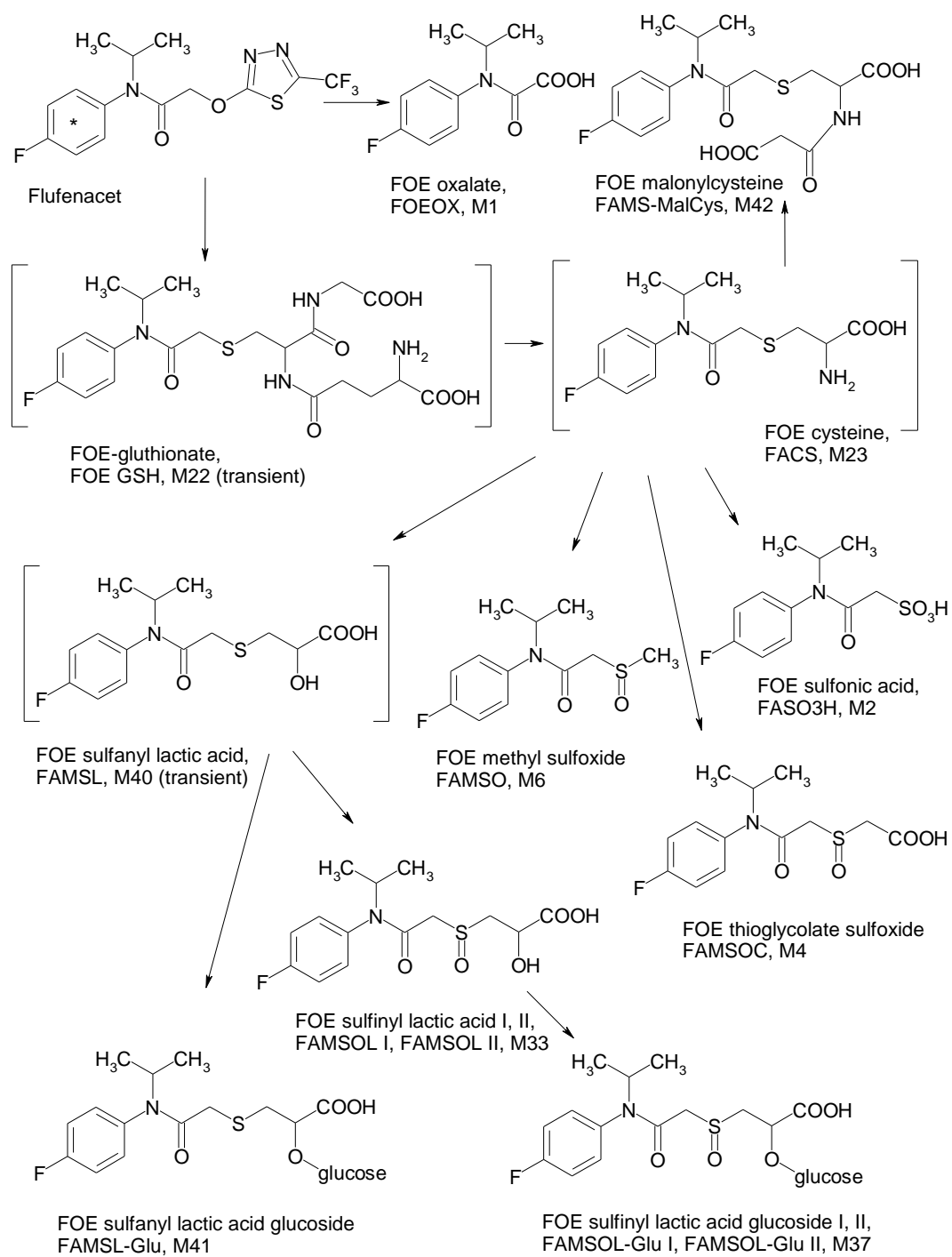
\* slight differences from TRR determination measured by combustion due to rounding of subfractions

**Table 7.2.1.2-9: Composition of residues in corn forage and fodder treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 1.46 kg as/ha**

Agricultural commodity	Corn forage		Corn fodder	
Days after treatment	82		129	
TRR [mg equ/kg]	0.62		1.91	
Metabolites released by MeOH at RT, MeOH refluxing and 1 N HCl at RT	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	-	-	1	0.02
FOE oxalate (FOEOX, M1)	27	0.17	22	0.42
Unknown 2	-	-	4	0.08
Unknown 3	-	-	1	0.02
FOE sulfinyl lactic acid glucoside I, II (FAMSOL-Glu, M37)	6	0.03	18	0.34
FOE thioglycolate sulfoxide (FAMSOC, M4)	7	0.04	5	0.10
FOE sulfinyl lactic acid I (FAMSOL I, M33)	5	0.03	5	0.10
FOE sulfinyl lactic acid II (FAMSOL II, M33)	19	0.12	16	0.30
Unknown 4	3	0.02	3	0.06
FOE sulfonyl lactic acid glucoside (FAMSL-Glu, M41)	25	0.15	-	-
FOE malonylcysteine (FAMS-MalCys, M42)	-	-	16	0.30
Total	92	0.56	91	1.74
<b>Total identified</b>	89	0.54	82	1.56

**Table 7.2.1.2-10: Composition of residues in the methanol extract of corn grain treated with [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a use rate of 1.46 kg as/ha**

Agricultural commodity	Corn grain	
Days after treatment	129	
TRR [mg equ/kg]	0.11	
Metabolites extracted with MeOH at RT	[% of TRR]	[mg equ/kg]
FOE sulfonic acid (FASO3H, M2)	4	<0.01
FOE oxalate (FOEOX, M1)	-	-
FOE sulfinyl lactic acid glucoside I, II (FAMSOL-Glu I, M37)	23	0.02
FOE thioglycolate sulfoxide (FAMSOC, M4)	9	0.01
FOE sulfinyl lactic acid I (FAMSOL I, M33)	2	<0.01
FOE sulfinyl lactic acid II (FAMSOL II, M33)	2	<0.01
FOE methyl sulfoxide (FAMSO, M6)	7	0.01
Total	47	0.04
<b>Total identified</b>	47	0.04

**Figure 7.2.1.2-3: Proposed metabolic pathway of flufenacet in corn following post-emergent foliar application**

\*  $^{14}\text{C}$  radiolabel [ . ] transient

**B 7.2.1.3 Additional plant metabolism studies with [thiadiazole-5-<sup>14</sup>C]flufenacet**

As mentioned before the older metabolism studies of flufenacet on plants were conducted with [fluorophenyl-UL-<sup>14</sup>C]- and the [thiadiazole-2-<sup>14</sup>C]flufenacet. To complete the pattern of all potential metabolites and metabolic pathways additional metabolism studies were recently conducted with [thiadiazole-5-<sup>14</sup>C]flufenacet on potatoes, wheat and rotated crops. These studies have still not been evaluated by registration authorities. They are summarized and presented in the following.

**REMARK ABOUT FORMATION OF TRIFLUOROACETATE (TFA) UNDER ENVIRONMENTAL AND PHYSIOLOGICAL CONDITIONS**

Metabolism studies of [thiadiazole-5-<sup>14</sup>C]flufenacet in primary and confined rotational crops often indicate trifluoroacetate (TFA) as a major metabolite. This metabolite is denoted misleadingly as trifluoroacetic acid, although the matrix of its formation (soil) or crops following uptake *via* the roots do not get acidic.

Under physiological and environmental conditions metabolic formation of TFA does not result in trifluoroacetic acid (TFA-H), rather than in formation of a trifluoroacetate salt (consists of TFA anion and counter cation). This is because of the very high acidity of TFA-H as characterized by its low pKa of 1.3<sup>12</sup> (for comparison, pKa of acetic acid: 4.76) indicating complete dissociation at higher pH.

During metabolic formation of TFA the acidity of the forming matrix (e.g. soil with microorganisms) does not change indicating that TFA cannot be present as carboxylic acid TFA-H. The dissociating proton of the carboxylic acid is immediately captured and neutralized by soil constituents due to the high buffer capacity of the soil. In its acid form it would damage the roots of plants rather than be taken up.

TFA is formed as trifluoroacetate anion with an undefined counter cation depending on the environment. Since the counter cation is undefined the TFA is usually denoted by the name of its parent acid, trifluoroacetic acid, keeping in mind that their salts are meant.

While the acid TFA-H is known to be highly irritant due to its high acidity, the TFA anion combined with an environmentally appearing cation behaves like an inert salt. Therefore, toxicological evaluation must not be conducted with TFA-H, but with a TFA salt.

**POTATO**

<b>Report:</b>	<b>KCA 6.2.1/06</b> , Bongartz, R.; 2012; M-441506-02-1
<b>Title:</b>	Metabolism of [thiadiazole-5- <sup>14</sup> C]Flufenacet in Potatoes
<b>Document No:</b>	M-441506-02-1
<b>Report No:</b>	EnSa-12-0537, dated 2012-12-10
<b>Guidelines:</b>	OECD guideline 501: Metabolism in Crops, adopted 8-January-2007, US EPA OCSPP Residue Chemistry Guideline OPPTS 860.1300
<b>GLP</b>	Yes; deviations: none

**Executive Summary**

The metabolism of [thiadiazole-5-<sup>14</sup>C]flufenacet was investigated in potatoes after pre-emergent application at a rate of approximately 630 g as/ha to the soil where seed potatoes have been planted one day before application. This use rate exceeded the intended field rate of 600 g as/ha by 5%. At maturity, 112 days after application, the potato plants were harvested, separated into tubers and foliage (leaves and stems), radioassayed for the level of total radioactive residues (TRR) and analyzed for the nature of these residues. In potato tubers, TRR amounted to 0.867 mg parent equivalents/kg (mg equ/kg) and in foliage to

<sup>12</sup> Winkler, S., 2011: Trifluoro acetic acid (AE C502988): Determination of the dissociation constant in water, unpublished report 20100672.02 of Siemens Prozess-Sicherheit, Frankfurt, Germany, for Bayer CropScience, Comp. No. M-418628-01-1

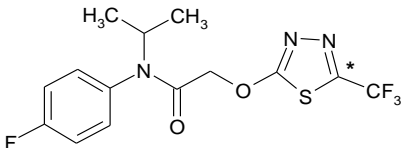
40.52 mg equ/kg. Extraction of these residues with acetonitrile/water (8/2, v/v) was nearly complete amounting to 99% or 98% of TRR in tubers or foliage, respectively.

The predominant portion of the residues consisted of  $^{14}\text{C}$ -labelled trifluoroacetate (TFA) contributing to 92% of TRR (corresponding to 0.801 mg equ/kg) in tubers and to 90% of TRR (corresponding to 36.45 mg equ/kg) in foliage. FOE-thiadone-glycoside was additionally detected as minor metabolite amounting to 1.8% of TRR (corresponding to 0.015 mg equ/kg) in tubers and to 4.4% of TRR (corresponding to 1.80 mg equ/kg) in foliage. Five additional unknown metabolites were also detected at a very low level, the sum of them accounted for 3.9% of TRR (corresponding to 0.034 mg equ/kg) in tubers and for 3.5% of TRR (corresponding to 1.41 mg equ/kg) in foliage. The portion of non-extractable residues was negligible amounting to 1% of TRR in tubers and 2% of TRR in foliage. The parent substance flufenacet was not observed in tubers or foliage.

From these results it was concluded that the thiadiazole ring is cleaved from the parent molecule and absorbed by the potato plants at a very low extent followed by formation of a glycoside conjugate. However, the predominant metabolic pathway proceeds via extensive degradation of the thiadiazole ring to form TFA that is widely taken up by the potato plants and translocated mainly into the green parts of the plants. A metabolic pathway is proposed in Figure 7.2.1.3-1.

## Material and Methods

### Test Material

Structural formula	 <p>* denotes the <math>^{14}\text{C}</math> label</p>
Chemical name	<p><i>N</i>-(4-Fluoro-phenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	$\text{C}_{14}\text{H}_{13}\text{F}_4\text{N}_3\text{O}_2\text{S}$
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5- $^{14}\text{C}$ ]Flufenacet
Specific radioactivity	1.9 MBq/mg (used in the study; the original test substance had a specific radioactivity of 3.81 MBq/mg or 103.04 $\mu\text{Ci}/\text{mg}$ )
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

### Test Plants

Test plant	Potato
Variety	Cilena
Growth stage at application	Soil treatment one day after seeding of the tubers and before emergence of the plants
Harvested commodities	Mature tubers (BBCH 97 – 99) together with potato vines

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Planting of seed potatoes, preparation and application of the spray mixture

A plant container (surface area 1 m<sup>2</sup>) was filled with a sandy loam soil (67% sand, 18% silt, 15% clay, 1.2% organic carbon, pH 6.9 (CaCl<sub>2</sub>)). Six seed potato tubers were planted in the soil one day before application of the spray mixture to the soil.

The original radiolabelled test substance was diluted with non-labelled flufenacet resulting in a specific radioactivity of 1.9 MBq/mg. Addition of a blank formulation yielded a SC 500 formulation with a concentration of the active substance of 42.4% (w/w). Addition of water finally resulted in the spray mixture of a volume of 104.5 mL.

The spray solution was applied to the bare soil surface of the prepared plant container using a computer controlled track sprayer fitted with a flat fan nozzle. The actual application rate amounted to 631 g as/ha being 5% higher than the intended field rate of 600 g as/ha. The stability the test substance in the spray mixture was demonstrated by radio-HPLC before and after application.

Cultivation of the test plants

The treated plant container was placed in an open vegetation hall with a glass roof and the plants were grown under outdoor conditions between April and August 2011. The mean temperatures ranged from 16 to 22°C and the mean sunshine periods between 83 to 231 hours/month.

Harvest and processing of the potatoes

Mature potato plants (BBCH 97 – 99) were dogged out of the soil 112 days after application of the test substance. The plants were separated into tubers and foliage (leaves and stems). Soil adhering to the tubers was removed after air-drying. Afterwards the tubers were washed with water, cut into slices and homogenized under liquid nitrogen using a high-speed stirrer (Polytron). Potato foliage was also homogenized as done with the tubers. Aliquots of the homogenates were extracted and the remaining homogenates stored at ≤ -18°C. The tuber wash, the extracts and the extracted solids were radioassayed.

Radioassaying, extraction and analysis of the plant samples

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed <sup>14</sup>CO<sub>2</sub> absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.002 mg equ/kg.

Homogenized plant samples were extracted three times with acetonitrile/water (8/2, v/v) using a high speed stirrer (Polytron) followed by one extraction with pure acetonitrile. The radioactivity contents of the extracts and the remaining solids (and in case to tuber the tuber wash) were numerically summarized to yield the total radioactive residues (TRR) of the original sample. The extracts were combined, concentrated and analysed for metabolite profiling by radio-HPLC and radio-TLC (TLC only done for polar HPLC fractions).

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5 µm particle size) operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The HPLC system was equipped with a UV detector (254 nm) and a radiomonitor with a glass scintillator (cell size 370 µL). Column recovery (97.9% for tuber analysis) was proven by comparison of the eluted and injected radioactivity. The LOQ for HPLC determination was derived from the background noise and the smallest radio-peak of the respective sample. HPLC-LOQs for tuber and foliage samples were set to 0.004 and 0.07 mg equ/kg. Radiolabelled parent substance, trifluoroacetate (isolated and identified in a metabolism study on rotated crops<sup>13</sup>) and FOE thiadone glycoside (isolated and identified in a metabolism study on wheat) as well as non-labelled FOE-thiadone were used as reference standards for co-chromatography.

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<sup>13</sup> Bongartz, R. (2012): Metabolism of [thiadiazole-5-<sup>14</sup>C]Flufenacet in Confined Rotational Crops, unpublished report EnSa-12-0535 of Bayer CropScience AG, Comp. No. M-443538-01-1.

One-dimensional radio-TLC was conducted on a silica gel TLC plates (20 x 20 cm, layer thickness 0.25 mm). Development of the spotted plates was performed with a solvent mixture consisted of ethyl acetate/2-propanol/water/acetic acid (65/24/11/1, v/v/v/v) after chamber saturation. The radioactive spots on the developed plates were visualized and quantified using a Bio-Imaging Analyzer.

LC-MS was conducted on a combination of RP18-HPLC (operated with a gradient mixture of 0.1% formic acid in water and in acetonitrile) and an Orbitrap mass spectrometer using electro-spray for ionization.

Potato tubers or foliage samples were extracted 13 or 22 days after harvest and storage at  $\leq -18^{\circ}\text{C}$ . The extracts were chromatographically analyzed for the composition of residues within one day after extraction.

## Findings

### Total radioactive residues

Seed potatoes were planted one day before application and matured potatoes were harvested 112 days after application of [thiadiazole-5- $^{14}\text{C}$ ]flufenacet to soil at a use rate of 631 g as/ha. The total radioactive residues (TRR) in the harvested tubers amounted to 0.867 mg parent equivalents/kg (mg equ/kg) and in foliage to 40.52 mg equ/kg. Very low radioactivity could be washed from the surface of the tubers with water amounting to 0.1% of TRR and corresponding to 0.001 mg equ/kg.

### Extraction of residues from potato tubers and foliage

The radioactive residues could be extracted almost completely using acetonitrile/water (8/2, v/v). A portion of 98.0% of TRR (corresponding to 0.850 mg equ/kg) was extracted from the tubers and 97.8% of TRR (corresponding to 39.66 mg equ/kg) from the foliage. In turn, the non-extractable portion accounted for 1.0% of TRR in tubers and 2.1% of TRR in foliage. The procedural losses were  $\leq 1\%$  of the respective TRR. Summing up these portions the resulting mass balance was complete for tubers and foliage.

### Nature of residues in potato tubers and foliage (Table 7.2.1.3-1).

The residues extracted from the tubers and foliage was analyzed by radio-HPLC on a reversed phase and radio-TLC on a straight phase and thus using two different chromatographic separation mechanisms. The main portion of the radiolabelled residues comprised of  $^{14}\text{C}$ -trifluoro acetate (TFA, M45) accounting for 90% of TRR (corresponding to 36.45 mg equ/kg) in the foliage and 92% of TRR (corresponding to 0.801 mg equ/kg) in the tubers. FOE-thiadone-glycoside (M25) appeared as minor metabolite amounting to 1.8% of TRR (corresponding to 0.015 mg equ/kg) in tubers and to 4.4% of TRR (corresponding to 1.80 mg equ/kg) in foliage. The parent substance flufenacet was not present in tubers and foliage. Five minor unknown metabolites accounted in sum to 3.9% of TRR in tubers and to 3.5% of TRR in foliage. The non-extractable portion of residues was negligible accounting for 1% of TRR in tubers and for 2% of TRR in the foliage.

## Conclusion

Seed potatoes were planted into soil. One day after planting [thiadiazole-5- $^{14}\text{C}$ ]flufenacet was applied to the soil surface at a use rate of approximately 630 g as/ha in the pre-emergence stage. Following cultivation till maturity the plants were harvested and analyzed for the composition of radiolabelled residue in tubers and foliage. The predominant portion of these residues consisted of  $^{14}\text{C}$ -labelled trifluoroacetate (TFA, M45). TFA amounted to  $\geq 90\%$  of TRR in both tubers (corresponding to 0.801 mg equ/kg) and foliage (corresponding to 40.52 mg equ/kg). A minor metabolite FOE-thiadone-glycoside (M25) appeared also in tubers and foliage at a portion of less than 5% of TRR. The unchanged parent substance was not detected in potatoes.

Obviously, the thiadiazole ring was split off of the parent substance, taken up by the potato plants at a very low extent and conjugated to a glycoside. However the main metabolic pathway proceeded by an extensive degradation of the thiadiazole ring to form TFA (M45) that is widely absorbed by potato plants and translocated particularly into the foliage. The metabolic pathway is proposed in Figure 7.2.1.3-1.

**Table 7.2.1.3-1: Composition of the radioactive residues in potatoes after pre-emergence application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 630 g as/ha to soil**

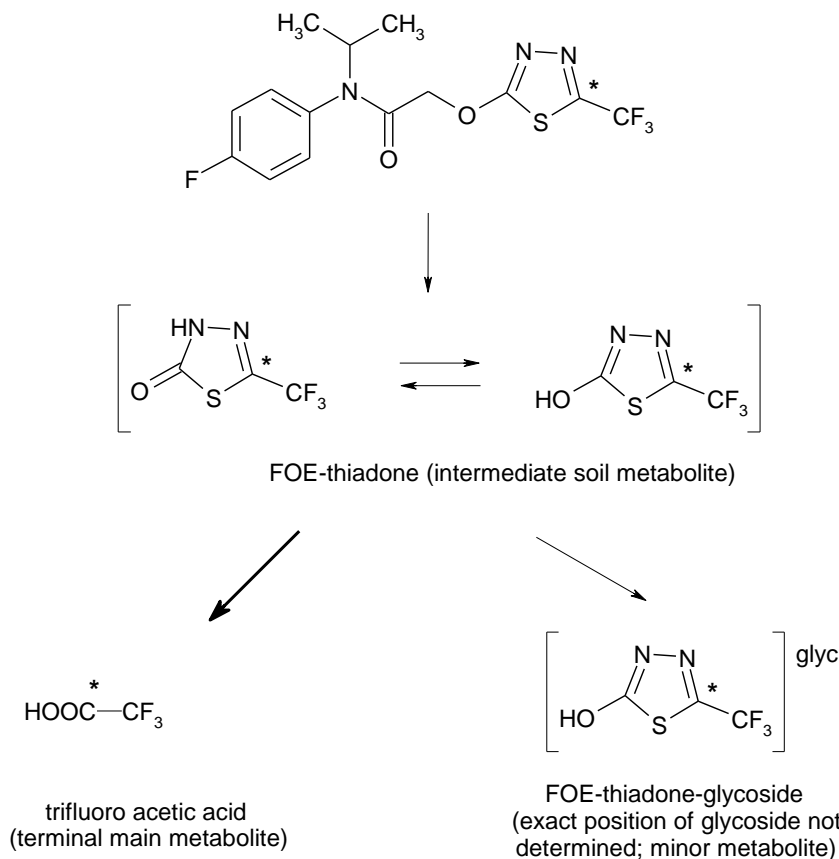
Potato	Tubers		Foliage	
	TRR = 0.867 mg equ/kg		TRR = 40.52 mg equ/kg	
	% of TRR	mg/kg <sup>#</sup>	% of TRR	mg/kg <sup>#</sup>
TFA (trifluoroacetic acid)	92.3	0.801	90.0	36.45
FOE-thiadone-glycoside	1.8	0.015	4.4	1.80
Total identified	94.1	0.816	94.4	38.25
Unknown 1	0.6	0.005	---	---
Unknown 2	---	---	0.7	0.28
Unknown 3	1.6	0.014	2.5	0.99
Unknown 4	0.8	0.007	0.3	0.13
Unknown 5	0.9	0.008	---	---
Total characterised*	3.9	0.034	3.5	1.41
Tuber wash	0.1	0.001	---	---
Procedural loss	0.8	0.007	<0.1	0.01
Total extractable	99.0	0.858	97.9	39.67
Non-extractable (PES) **	1.0	0.009	2.1	0.85
Accountability	100.0	0.867	100.0	40.52

\* The non-identified compounds were characterised by their extraction and chromatographic behaviour.

\*\* PES = post extraction solids

# mg/kg means mg parent equivalents/kg

**Figure 7.2.1.3-1: Metabolic pathway of [thiadiazole-5-<sup>14</sup>C]flufenacet in potatoes after pre-emergent application to soil at a use rate of 630 g as/ha**



\* position of  $^{14}\text{C}$ -label

## WHEAT

<b>Report:</b>	<b>KCA 6.2.1/07</b> , Bongartz, R.; Miebach, D.; 2013; M-444475-01-1
<b>Title:</b>	Metabolism of [thiadiazole-5- <sup>14</sup> C]Flufenacet in Wheat
<b>Document No:</b>	M-444475-01-1
<b>Report No:</b>	EnSa-12-0536, dated 2013-01-07
<b>Guidelines:</b>	OECD Guideline 501: Metabolism in Crops, adopted 8-January-2007, US EPA OCSPP Residue Chemistry Guideline OPPTS 860.1300
<b>GLP</b>	Yes; deviations: none

## Executive Summary

The metabolism of [thiadiazole-5-<sup>14</sup>C]flufenacet was investigated in wheat following a foliar treatment at a use rate of 270 g as/ha in the mid-tillering growth stage. This use rate exceeded the intended field rate of 240 g as/ha by 12.5%. The total amount and the nature of residues was disclosed in wheat forage sampled four days after treatment (DAT), in wheat hay sampled 56 DAT and in wheat straw and grain harvested 84 DAT, respectively.

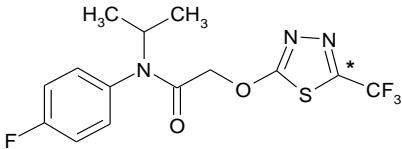
The total radioactive residues (TRR) amounting to 5.145 mg equ/kg in forage, 2.689 mg equ/kg in hay, 2.974 mg equ/kg in straw and to 0.704 mg equ/kg in wheat grain could almost completely be extracted with acetonitrile/water (8/2, v/v, 4x) at room temperature followed by extraction with acetonitrile/water (1/1, v/v) plus formic acid at elevated temperatures.

Whereas the parent substance flufenacet revealed to be the prominent residue component in wheat forage it was almost completely metabolized in wheat hay and straw and was no more detectable in wheat grain. The metabolite trifluoroacetate (TFA) was still not detected in wheat forage, but proved to be the main residue component in wheat and straw. In grain, practically the complete radioactive residues consisted of radiolabelled TFA. An intermediate metabolite, FOE-thiadone-glycoside, appeared already in the forage commodity at a relevant portion and increased slightly to approximately one third of the total residues in hay and straw, but was almost completely degraded to TFA in grain. The portion of non-extractable residues was negligible in all wheat commodities not exceeding 1% of TRR.

Obviously, the thiadiazole ring was rapidly split off of the parent substance and conjugated to a glycoside to a moderate extent. The further metabolic pathway proceeded in an extensive degradation of the thiadiazole ring to form TFA as the main residue component in hay and straw and as the terminal and nearly exclusive residue component in wheat grain. The metabolic pathway is proposed in Figure 7.1.2.3-2.

## Material and Methods

## Test Material

Structural formula	 <p>* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluoro-phenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043

Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5- <sup>14</sup> C]Flufenacet
Specific radioactivity	1.9 MBq/mg (used in the study; the original test substance had a specific radioactivity of 3.81 MBq/mg or 103.04 µCi/mg)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

### Test Plants

Test plant	Spring wheat
Variety	Thasos
Growth stage at application	Post-emergent foliar application at growth stage BBCH 21 – 25 (beginning of first tillering – five tillers detectable)
Harvested commodities	Wheat forage (BBCH 29, end of tillering), PHI: 4 days Wheat hay (BBCH 75-83, medium milk – early dough stage), PHI: 56 days Wheat grain and straw (BBCH 89, full ripe grain), PHI: 84 days

### Sowing of wheat, preparation and application of the spray mixture

A plant container (surface area 1 m<sup>2</sup>) was filled with a sandy loam soil (67% sand, 18% silt, 15% clay) having an organic carbon content of 1.2% and a pH (CaCl<sub>2</sub>) of 6.9. Wheat was sown in 10 rows at a sowing density of approximately 500 seeds/m<sup>2</sup>.

The original radiolabelled test substance was diluted with non-labelled flufenacet resulting in a specific radioactivity of 1.9 MBq/mg. Addition of a blank formulation yielded a SC 500 formulation with a concentration of the active substance of 42.4% (w/w). Addition of water finally resulted in the spray mixture of a volume of 105 mL.

The spray mixture was sprayed to the wheat plants grown in the plant container using a computer controlled track sprayer fitted with a flat jet nozzle at the mid tillering growth stage BBCH 21 - 25. The actual application rate amounted to 270 g as/ha, being 12.5% higher than the intended field rate of 240 g as/ha. The stability the test substance in the spray mixture was demonstrated by radio-HPLC before and after application.

### Cultivation of the test plants

The treated plant container was placed in an open vegetation hall with a glass roof and the plants were grown under outdoor conditions between April and August 2011. During sunshine periods the glass roof was opened. The mean temperatures ranged from 16 to 22°C and the mean sunshine periods between 83 to 231 hours/month. Commercial cereals fungicides and insecticides were applied when required according to agricultural practice.

### Harvest and processing of the wheat commodities

Wheat forage (BBCH 29): The plants of two of the ten rows were cut above the soil, cut into small pieces and homogenized under liquid nitrogen with use of a high-speed stirrer (Polytron). An aliquot of the homogenate was extracted and the remaining material stored at ≤ -18°C.

Wheat hay (BBCH 75 – 83): The plants of another two rows were cut above the soil, dried for four days at room temperature, cut into small pieces and homogenized and stored as mentioned for wheat forage.

Wheat straw and grain (BBCH 89): The remaining plants were cultivated until full maturity and then cut above the soil. The seeds were pulled out the ears by hand yielding the grain sample. The remaining ears and chaffs were combined with the straw and cut into small pieces. Grain and straw were separately homogenized under liquid nitrogen and stored as described for wheat hay.

### Radioassaying, extraction and analysis of the plant samples

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were first combusted and the formed  $^{14}\text{CO}_2$  absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.002 mg parent equivalents/kg (0.002 mg equ/kg).

Immature homogenized plant samples were extracted three times with acetonitrile/water (8/2, v/v) using a high speed stirrer (Polytron) followed by one extraction with pure acetonitrile (conventional extraction). Wheat hay, straw and grain were successively extracted with acetonitrile/water (1/1, v/v) and acetonitrile/water (1/1, v/v) plus formic acid at elevated temperatures with microwave assistance (exhaustive extraction) to complete the extraction. The radioactivity contents of the extracts and the remaining solids were numerically summarized to yield the total radioactive residues (TRR) of the original sample. The conventional and exhaustive extracts were separately combined, concentrated and analysed for metabolite profiling by radio-HPLC and radio-TLC (TLC only done for polar HPLC fractions).

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5  $\mu\text{m}$  particle size) operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The HPLC system was equipped with a UV detector (254 nm) and a radiomonitor with a glass scintillator (cell size 370  $\mu\text{L}$ ). Column recovery was proven by comparison of the eluted and injected radioactivity. It was excellent amounting to 97.6 – 99.6% for analysis of forage, hay, straw and grain extract. The LOQ for HPLC determination was derived from the background noise and the smallest radio-peak of the respective sample. HPLC-LOQs for the different extracts were set to 0.002 (grain) to 0.018 (forage) mg equ/kg. Radiolabelled parent substance, trifluoroacetate (isolated and identified in a metabolism study on rotated crops and non-labelled FOE-thiadone were used as reference standards for co-chromatography).

One-dimensional radio-TLC was conducted on a silica gel TLC plates (20 x 20 cm, layer thickness 0.25 mm). Development of the spotted plates was performed with a solvent mixture consisted of ethyl acetate/2-propanol/water/acetic acid (65/24/11/1, v/v/v/v) after chamber saturation. The radioactive spots on the developed plates were visualized and quantified out using a Bio-Imaging Analyzer.

LC-MS of parent flufenacet and FOE-thiadone-glycoside was conducted on a combination of RP18-HPLC (operated with a gradient mixture of 0.1% formic acid in water and in acetonitrile) and an Orbitrap mass spectrometer using electro-spray for ionization.

Wheat samples (forage, hay, straw and grain) were extracted one to twelve days after harvest and storage at  $\leq -18^\circ\text{C}$ . The extracts were chromatographically analyzed for the composition of residues within one to two days after extraction.

## **Findings**

### Total radioactive residues

Spring wheat was sprayed with [thiadiazole-5- $^{14}\text{C}$ ]flufenacet at a use rate of 270 g as/ha in the mid tillering growth stage. Plant commodities were samples after different intervals after treatment: forage 4 days, hay 56 days, straw and grain 84 days. The total radioactive radioactivity (TRR) in these commodities amounted to 5.145 mg equ/kg in forage, 2.689 mg equ/kg in hay, 2.974 mg equ/kg in straw and 0.704 mg equ/kg in grain.

### Extraction of residues from wheat commodities (Table 7.2.1.3-2)

The radioactive residues could be extracted almost completely from all wheat commodities using acetonitrile/water (8/2, v/v; “conventional extraction”) at room temperature and acetonitrile/water (1/1, v/v, partly with formic acid; “exhaustive extraction”) at elevated temperature. The totally extractable residues amounted to 99.0 – 99.7% of TRR. In turn, the non-extractable portion accounted for 0.3 – 1.0% of TRR. The prominent portion of residues could already be extracted at room temperature ranging from 75.2% of TRR (grain) to 98.3% of TRR (forage).

Nature of residues in wheat commodities (Table 7.2.1.3-3)

The residues extracted from the wheat forage, hay, straw and grain were analyzed by radio-HPLC on a reversed phase and radio-TLC on a straight phase and thus using two different chromatographic separation mechanisms.

The parent substance flufenacet was the main residue component in wheat forage sampled four days after application. It amounted to 76.7% of TRR (3.944 mg/kg). However, flufenacet was almost completely metabolised in hay and straw ( $\leq 1.8\%$  of TRR) and did no longer appear in wheat grain.

Trifluoroacetate (TFA, M45) did still not appear in wheat forage, but proved to get the main residue component in hay (63.1% of TRR; 1.697 mg equ/kg) and straw (61.7% of TRR; 1.836 mg equ/kg). In wheat grain, almost the total residues consisted of TFA (M45) amounting to 99.2% of TRR corresponding to 0.698 mg equ/kg.

FOE-thiadone-glycoside (M25) was already formed in wheat forage, four days after application of flufenacet, amounting to 21.6% of TRR (1.113 mg equ/kg). In hay and straw, it contributed to approximately one third of the total residues (30.5 – 33.5% of TRR, corresponding to 0.822 mg equ/kg in hay and to 0.997 mg equ/kg in straw). It decreased to a very minor metabolite in wheat grain accounting for 0.4% of TRR (0.003 mg equ/kg).

**Conclusion**

Following foliar treatment of spring wheat with [thiadiazole-5- $^{14}\text{C}$ ]flufenacet at a use rate of 270 g as/ha the radioactive residues were investigated in wheat forage sampled four days after treatment (DAT), in wheat hay sampled 56 DAT and in wheat straw and grain harvested 84 DAT.

Whereas the parent substance flufenacet revealed to be the prominent residue component in wheat forage it was almost completely metabolized in wheat hay and straw and was no more detectable in wheat grain. The metabolite trifluoroacetate (TFA, M45) could still not be detected in wheat forage, but proved to be the main residue component in wheat and straw. In grain, practically the complete radioactive residues consisted of radiolabelled TFA. An intermediate metabolite, FOE-thiadone-glycoside (M25), appeared already in the forage commodity at a relevant portion and increased slightly to approximately one third of the total residues in hay and straw, but was almost completely degraded to TFA in grain.

Obviously, the thiadiazole ring was rapidly split off of the parent substance and conjugated to a glycoside at a moderate extent. The further metabolic pathway proceeded in an extensive degradation of the thiadiazole ring to form TFA as the main residue component in hay and straw and as the terminal and nearly exclusive residue component in wheat grain. The metabolic pathway is proposed in Figure 7.2.1.3-2.

**Table 7.2.1.3-2: Extractability of radioactive residues from wheat commodities after foliar application of [thiadiazole-5- $^{14}\text{C}$ ]flufenacet at a use rate of 270 g as/ha**

Wheat	Forage, 4 DAT <sup>##</sup>		Hay, 56 DAT		Straw, 84 DAT		Grain, 84 DAT	
	% TRR	mg/kg <sup>#</sup>	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
TRR	100	5.145	100	2.689	100	2.974	100	0.704
Conventional extraction *	98.3	5.057	93.5	2.514	94.0	2.796	75.2	0.529
Exhaustive extraction **	--	--	5.9	0.157	5.2	0.155	24.4	0.172
Procedural loss	0.7	0.034	0.3	0.009	0.2	0.006	--	--
Total extractable	99.0	5.091	99.7	2.680	99.4	2.957	99.7	0.701
Non-extractable (PES) ***	1.0	0.053	0.3	0.009	0.6	0.017	0.3	0.002
Accountability	100.0	5.145	100.0	2.689	100.0	2.974	100.0	0.704

\* Extraction with acetonitrile/water (8/2, v/v) at room temperature

\*\* Succeeding extraction with acetonitrile/water (1/1, v/v) plus formic acid at elevated temperature

\*\*\* PES: post extraction solids

# mg/kg: mg parent equivalents/kg (mg equ/kg)

## 4 DAT: sampling 4 days after treatment

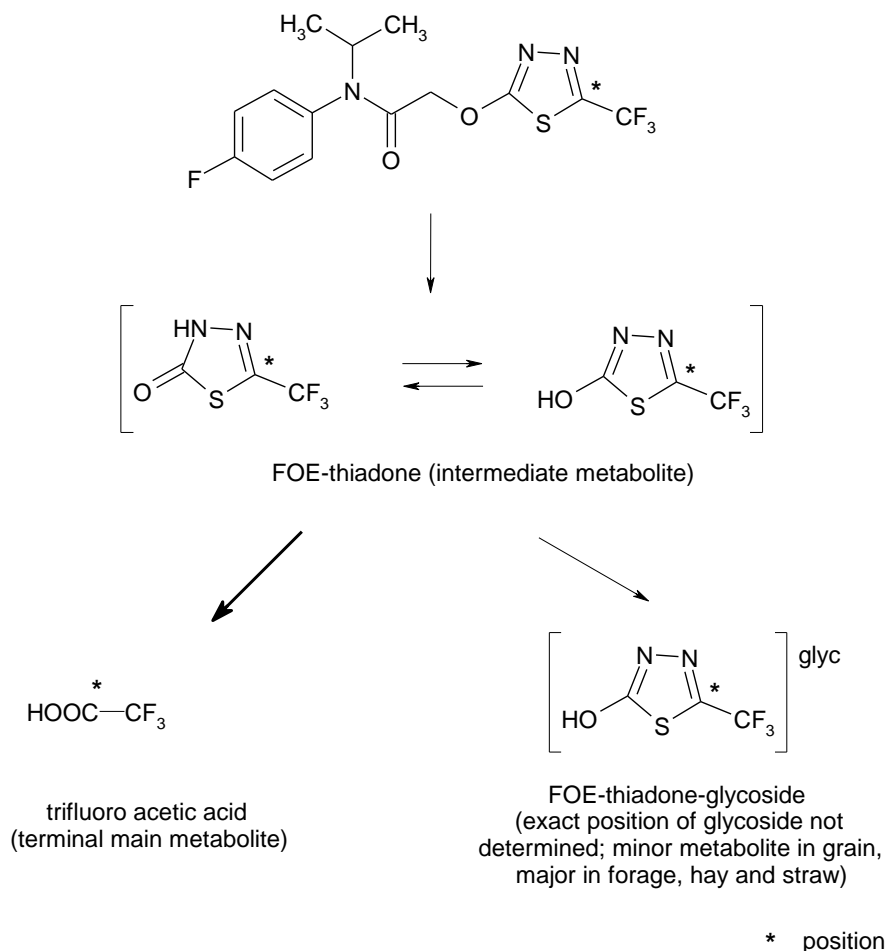
**Table 7.2.1.3-3: Composition of the radioactive residues in wheat commodities after foliar application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 270 g as/ha**

Wheat	Forage		Hay		Straw		Grain	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
<b>Conventional extraction</b> (at room temperature)								
Flufenacet	76.7	3.944	1.8	0.048	0.4	0.013	--	--
TFA (trifluoroacetate)	--	--	58.9	1.583	58.0	1.726	74.8	0.526
FOE-thiadone-glycoside	21.6	1.113	28.9	0.778	32.0	0.952	0.4	0.003
Unknown 1	--	--	--	--	0.6	0.019	--	--
Unknown 2	--	--	0.6	0.015	0.7	0.020	--	--
Unknown 3	--	--	0.4	0.011	--	--	--	--
Unknown 3	--	--	1.8	0.048	1.0	0.029	--	--
Unknown 5	--	--	0.6	0.017	1.3	0.038	--	--
Unknown 6	--	--	0.6	0.015	--	--	--	--
Procedural loss	0.7	0.034	0.3	0.009	0.2	0.006	--	--
<b>Exhaustive extraction</b> (at elevated temperature, performed after conventional extraction)								
TFA (trifluoroacetate)	--	--	4.2	0.114	3.7	0.110	24.4	0.172
FOE-thiadone-glycoside	--	--	1.6	0.044	1.5	0.045	--	--
<b>Summary of extraction</b>								
Flufenacet	76.7	3.944	1.8	0.048	0.4	0.013	--	--
TFA (trifluoroacetate)	--	--	63.1	1.697	61.7	1.836	99.2	0.698
FOE-thiadone-glycoside	21.6	1.113	30.5	0.822	33.5	0.997	0.4	0.003
<b>Total identified</b>	<b>98.3</b>	<b>5.057</b>	<b>95.4</b>	<b>2.566</b>	<b>95.7</b>	<b>2.845</b>	<b>99.7</b>	<b>0.701</b>
Total characterized *	--	--	3.9	0.106	3.6	0.106	--	--
Non-extractable (PES) **	1.0	0.053	0.3	0.009	0.6	0.017	0.3	0.002
<b>Accountability</b>	<b>100.0</b>	<b>5.145</b>	<b>100.0</b>	<b>2.689</b>	<b>100.0</b>	<b>2.974</b>	<b>100.0</b>	<b>0.704</b>

\* Characterized by the extraction and chromatographic behaviour

\*\* PES: post extraction solids

<sup>#</sup> mg/kg: mg parent equivalents/kg (mg equ/kg)

**Figure 7.2.1.3-2: Metabolic pathway of [thiadiazole-5-<sup>14</sup>C]flufenacet in wheat after foliar application at a use rate of 270 g as/ha**

### Summary of the metabolism of flufenacet in plants including the new metabolism studies

From the metabolism studies submitted for approval in the EU and USA a conclusion of a common metabolic pathway of flufenacet in plants was made. The initial metabolic reaction is a cleavage of the molecule into the thiadone and acetamide moiety by glutathione (GSH) conjugation of the acetamide part resulting in the transient glutathionate conjugated FOE GSH (M22).

This transient glutathione conjugate is further metabolized by splitting off glycine and glutamine acid yielding the FOE cysteine conjugate (M23). All further metabolites can be considered as hydrolysis, oxidation and conjugation products of the FOE cysteine conjugate. However, the FOE oxalate (M01) most likely arose through direct oxidation of a transient primary alcohol hydrolysis product of Flufenacet (FOE alcohol, M03).

Due to the initial cleavage of the parent molecule caused by glutathionate conjugation, trifluoromethyl thiadone (M09) was released. While this transient moiety was not observed, various conjugates were formed, the quantitatively most important being the corresponding *N*-glucoside (M 25). In soybeans, the malonylalanine conjugate (M34) predominated.

The additional studies with [fluorophenyl-UL-<sup>14</sup>C]flufenacet on potato (pre- and post-emergence application), wheat and corn (both post-emergence application) confirmed this metabolic pathway. Additional plant metabolism studies with [thiadiazole-5-<sup>14</sup>C]flufenacet in potato (pre-emergence application), wheat (post-emergence application) and in the rotational crops wheat, turnip and Swiss chard

disclosed an already known metabolite, a glycoside conjugate of FOE thiadone, probably THNG (M25), and a new metabolite, i.e. trifluoroacetate, TFA (denoted as the parent substance trifluoroacetic acid, since the counter cation depends from the surrounding medium, and therefore varies and is not defined). Trifluoroacetate proved to be the main residue component in all plant metabolism and confined rotational crop studies with the [thiadiazole-5-<sup>14</sup>C]-label. The combined metabolic pathway of flufenacet in plants is shown in Figure 7.2.1.2-5. In order to find common major metabolites as potential marker substances for a residue analytical method all major metabolites of flufenacet in all investigated plants are compiled in a summary that is presented in Table 7.2.1.3-4 (given in % of TRR) and Table 7.2.1.3-5 (given in mg equ/kg).

The parent substance flufenacet did not occur in any crop. The main flufenacet metabolites in corn, cotton, soybean, potato and wheat are marked in **bold** in the summary Table 7.2.1.3-4 and Table 7.2.1.3-5.

However, no metabolite can be found that proved to be major in all crops and can be selected as marker substance. Therefore, a common moiety method was developed as alternative method. Using the [fluorophenyl-UL-<sup>14</sup>C]-labelled flufenacet all the metabolites containing a common moiety, i.e. “*N*-(4-fluorophenyl)-*N*-isopropyl amine” are compiled in the [bluish array](#) of Table 7.2.1.3-4 and Table 7.2.1.3-5. Based on these metabolites the residue definition of flufenacet residues in plants was proposed as parent substance and all metabolites containing the common moiety. When summing up the metabolites with the common moiety the resulting sum represents the major portion of TRR in most of the examined raw agricultural commodities, except in corn kernels with no identified residues (Baird, 1994). This corn/maize study can be replaced by the study of Krolski and Bosnak (1998) conducted with the same plant species. Metabolites containing this common moiety are all located inside the [blue frame](#) in Figure 7.2.1.3-3.

Using flufenacet radiolabeled as [thiadiazole-2-<sup>14</sup>C] or [thiadiazole-5-<sup>14</sup>C] flufenacet this results in other label-specific metabolites derived from the thiadone ring of flufenacet ([highlighted in red](#)).

It can be concluded the common moiety approach seems to be an appropriate solution for deriving the residue definitions for enforcement and monitoring as well as for risk assessment purposes.

This approach (only when application is made pre- and early post-emergence) has been confirmed by EFSA in “Reasoned opinion on the review of the existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005”, EFSA Journal 2012;10(4):2689. Consequently the currently binding residue definition for food of plant origin (for monitoring and enforcement purposes) according to Commission Regulation (EU) No 1127/2014 of 20 October 2014 is “Flufenacet (sum of all compounds containing the *N* fluorophenyl-*N*-isopropyl moiety expressed as flufenacet)”. Residue definition for risk assessment purposes is proposed in the EFSA opinion to be the same. If in the future uses are needed where the application is closer to harvest the metabolism and residue definition would need to be reconsidered.

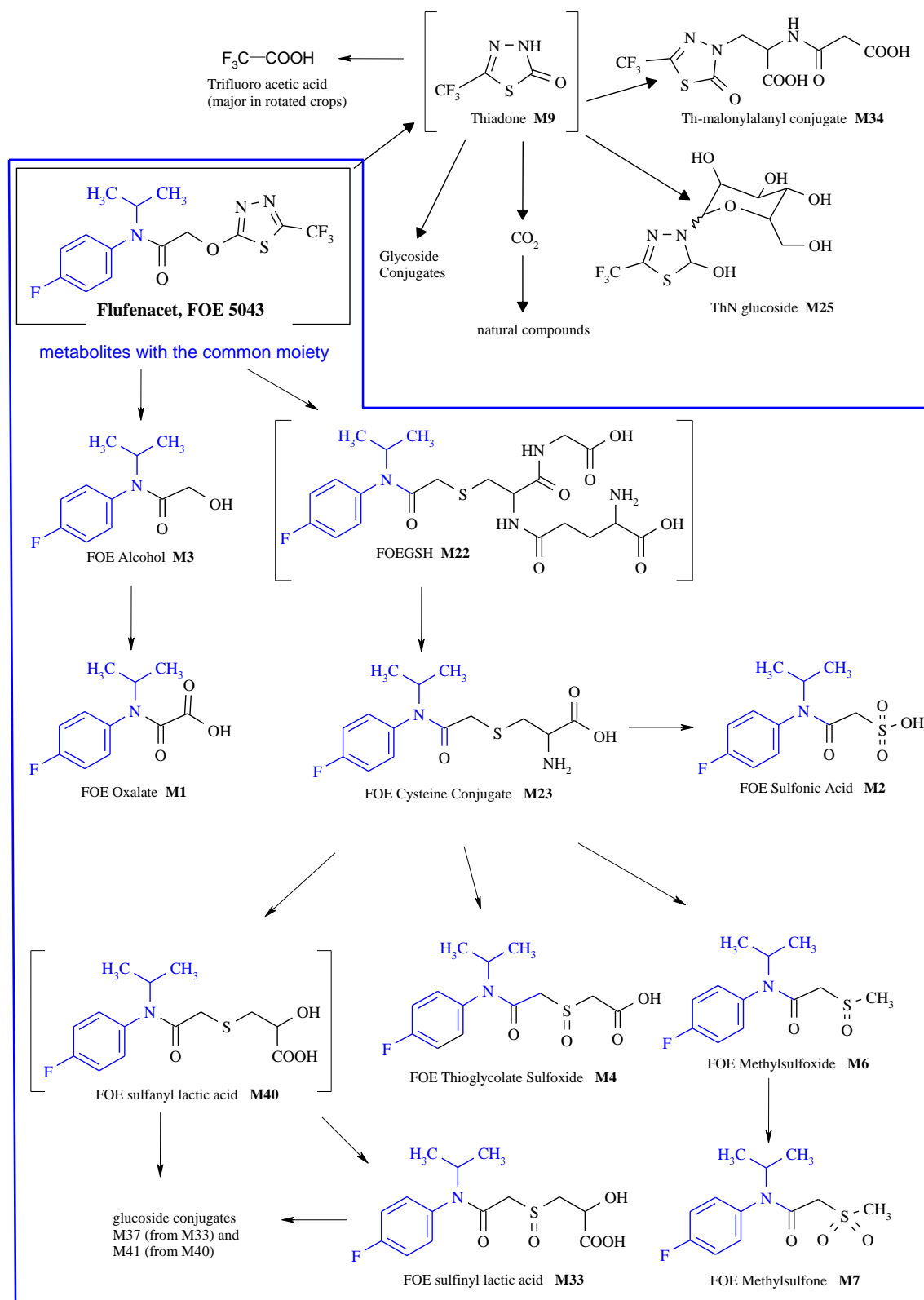
**Table 7.2.1.3-4: Metabolites of flufenacet in plant metabolism studies following pre- and post-emerg. application using three label positions (% TRR)**

Crop (radiolabel)	Corn (F-phenyl)		Cotton (F-phenyl)		Soybean (F-phenyl)		Soybean (thia-2)		Potato (F-phenyl)		Wheat (F-phenyl)		Corn (F-phenyl)		Potato (thia-5)		Wheat (thia-5)	
Appl Rate [kg as/ha]	1.370 (pre)		1.778 (pre)		1.485 (pre)		1.380 (pre)		2.58 (pre)	3.01 (post)	0.52 (post)		1.46 (post)		0.630 (pre)		0.270 (post)	
Agricultural Commodity	Ker- nels	Fod- der	Seeds	Total plant	Beans	Fo- rage	Beans	Hay	Tuber	Tuber	Grain	Straw	Grain	Fod- der	Tuber	Grai n	Straw	
TRR [mg equ/kg]	0.012	0.498	0.067	1.54	1.02	8.49	0.68	5.78	0.35	0.32	0.62	2.04	0.11	1.91	0.867	0.70 4	2.974	
A.S.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4	
M1, -oxalate	-	41	-	11	6	18					65	14	-	22				
M2, -sulfonic acid	-	5	-	66	5	42			-	4	-	15	4					
M4, -thioglycolate sulfoxide	-	11	-	6	26	17			-	7	-	7	9	5				
M6, -methyl sulfoxide	-	1	-	-	6	6			-	-	-	-	7	-				
M7, -methyl sulfone	-	3	-	2	4	9			-	-	-	-	-	-				
M33, -sulfinyl lactic acid I, II	-	9	-	-	-	-			-	-	-	35	4	21				
M37, -sulfinyl lactic acid glucoside I, II	-	-	-	-	-	-			-	-	-	3	23	18				
M41, sulfanyl lactic acid glucoside	-	-	-	-	-	-			19	17	-	-	-	-				
M23, -cysteine	-	-	-	-	-	-			44	52	-	-	-					
M42, malonyl cysteine	-	-	-	-	-	-			-	-	-	-	-	16				
M25, THNG							-	66							1.8	0.4	33.5	
M34, Th-malonyl- alanine							66	-							-	-	-	
Other Th- conjugates							-	13							-	-	-	
TFA							-	-							92.3	99.2	61.7	
Reference	Braid, 1994 M-002270-01-1		Krolski, Bosnak, 1995 M-002277-01-1		Krolski, Bosnak, 1995 M-002279-01-1				Beedle, Ying, 2000 M-020428-01-1		Krolski, Bosnak, 1997 M-002275-01-1		Krolski, Bosnak, 1998 M-005755-01-1		Bongartz Klankers, 2012 M-441506- 02-1		Bongartz, Miebach, 2013 M-444475-01- 1	

**Table 7.2.1.3-5: Metabolites of flufenacet in plant metabolism studies following pre- and post-emerg. application using three label positions (mg equ/kg)**

Crop (radiolabel)	Corn (F-phenyl)		Cotton (F-phenyl)		Soybean (F-phenyl)		Soybean (thia-2)		Potato (F-phenyl)		Wheat (F-phenyl)		Corn (F-phenyl)		Potato (thia-5)	Wheat (thia-5)	
Appl Rate [kg as/ha]	1.370 (pre)		1.778 (pre)		1.485 (pre)		1.380 (pre)		2.58 (pre)	3.01 (post)	0.52 (post)		1.46 (post)		0.630 (pre)	0.270 (post)	
Agricultural Commodity	Ker- nels	Fod- der	Seeds	Total plant	Beans	Fo- rage	Beans	Hay	Tuber	Tuber	Grain	Straw	Grain	Fod- der	Tuber	Grain	Straw
TRR [mg equ/kg]	0.012	0.498	0.067	1.54	1.02	8.49	0.68	5.78	0.35	0.32	0.62	2.04	0.11	1.91	0.867	0.704	2.974
A.S.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.4
M1, -oxalate	-	0.205	-	0.17	0.06	1.53					0.40	0.29	-	0.42			
M2, -sulfonic acid	-	0.026	-	1.02	0.05	3.57			-	0.01	-	0.31	-				
M4, -thioglycolate sulfoxide	-	0.056	-	0.09	0.27	1.44			-	0.02	-	0.14	0.01	0.10			
M6, -methyl sulfoxide	-	0.003	-	-	0.06	0.51			-	-	-	-	0.01	-			
M7, -methyl sulfone	-	0.016	-	0.03	0.04	0.76			-	-	-	-	-	-			
M33, -sulfinyl lactic acid I, II	-	0.045	-	-	-	-			-	-	-	0.71	-	0.40			
M37, -sulfinyl lactic acid glucoside I, II	-	-	-	-	-	-			-	-	-	0.06	0.02	0.34			
M41, sulfanyl lactic acid glucoside	-	-	-	-	-	-			0.07	0.05	-	-	-	-			
M23, -cysteine	-	-	-	-	-	-			0.15	0.17	-	-	-				
M42, malonyl cysteine	-	-	-	-	-	-			-	-	-	-	-	0.30			
M25, THNG							-	3.81							0.015	0.003	0.997
M34, Th-malonyl- alanine							0.44	-							-	-	-
Other Th- conjugates							-	0.75							-	-	-
TFA							-	-							0.801	0.698	1.836
Reference	Braid, 1994 M-002270-01-1		Krolski, Bosnak, 1995 M-002277-01-1		Krolski, Bosnak, 1995 M-002279-01-1				Beedle, Ying, 2000 M-020428-01-1		Krolski, Bosnak, 1997 M-002275-01-1		Krolski, Bosnak, 1998 M-005755-01-1		Bongartz Klankers, 2012 M- 441506-02 -1	Bongartz, Miebach, 2013 M-444475-01-1	

**Figure 7.2.1.3-3: Proposed metabolic pathway of flufenacet in plants, combination of all plant metabolism studies with three different radiolabels**



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**B.7.2.2. Livestock**

The nature of flufenacet residues in laying hen was investigated in the framework of Directive 91/414/EEC. The studies used [fluorophenyl-UL-<sup>14</sup>C]flufenacet, [thiadiazole-2-<sup>14</sup>C]flufenacet and [fluorophenyl-UL-<sup>14</sup>C]flufenacet oxalate, the latter one being the main plant metabolite in poultry and ruminant feed. The studies were reviewed in the Monograph.

The nature of flufenacet residues in goat was investigated in the framework of Directive 91/414/EEC. The studies used [fluorophenyl-UL-<sup>14</sup>C]flufenacet, [thiadiazole-2-<sup>14</sup>C] flufenacet, [fluorophenyl-UL-<sup>14</sup>C]flufenacet oxalate and [thiadiazole-2-<sup>14</sup>C]thiadone-*N*-glucoside, the later two substances being the main plant metabolites in ruminant feed. All studies except the one using [thiadiazole-2-<sup>14</sup>C]thiadone-*N*-glucoside were reviewed in the Monograph.

In the EFSA reasoned opinion a detailed assessment is provided on the review of the existing maximum residue levels according to Art 12 of Regulation (EC) no. 396/2005 (2012). The general metabolic pathways in rodents and livestock were found to be comparable.

The metabolism of [thiadiazole-2-<sup>14</sup>C]thiadone-*N*-glucoside in the lactating goat was performed on request of the US EPA. It was not submitted with the former EU application. Therefore it is summarized in this submission.

Since the parent compound degrades rapidly in plants and is not detectable in animal feeding items the metabolism study using [fluorophenyl-UL-<sup>14</sup>C] FOE oxalate provides the most relevant information. Oral administration of [fluorophenyl-U-<sup>14</sup>C]flufenacet oxalate to ruminant and poultry showed its metabolic stability. Flufenacet oxalate is essentially not metabolised by the animal. The low residue levels in tissue, milk and eggs suggest that flufenacet oxalate is minimally absorbed and rapidly excreted. This metabolic stability was confirmed by a bio-availability study of flufenacet oxalate in rats<sup>14</sup>. Following oral administration of radiolabeled flufenacet oxalate to three rats at a dose rate of approx. 1 mg/kg bw 19 – 37% of the dose was excreted with urine and 61 – 80% was excreted with faeces as unchanged flufenacet oxalate.

The metabolism studies performed with flufenacet indicate a wide range of metabolites are formed containing the *N*-fluorophenyl-*N*-isopropyl moiety. Therefore, EFSA concluded that for commodities of animal origin, it is desirable to include all metabolites containing the *N*-fluorophenyl-*N*-isopropyl moiety in the residue definition, both for enforcement and risk assessment.

New plant metabolism studies with [thiadiazole-5-<sup>14</sup>C]flufenacet in primary and succeeding plants revealed trifluoroacetate (M45) as a major metabolite in edible plant parts and in plant parts intended as feeding stuff for livestock animals. For a complete dietary risk assessment including residues in food of animal origin, a potential residue transfer of trifluoroacetate from feeding stuff to food of animal origin has been investigated. Therefore, metabolism studies on <sup>14</sup>C-labelled trifluoroacetate in goat and hen were conducted.

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<sup>14</sup> Part of the study of [REDACTED] (1995): Metabolism of FOE 5043 in Soybeans, unpublished report 105187 of Miles Inc. Kansas, USA, now Bayer CropScience, Comp. No. M-002278-01-1.

**B 7.2.2.1 Poultry**

An overview on the metabolism studies on laying hen is presented in Table 7.2.2.1-1.

**Table 7.2.2.1-1: Overview of hen metabolism studies with <sup>14</sup>C-label flufenacet**

Animal	Label	Report	Submission	
			EU baseline dossier, Annex II , Section 4, Point 6	Reported in supplementary dossier Section 6
laying hen	[Fluorophenyl-UL- <sup>14</sup> C] FOE 5043	██████████ 1995; M-002251-01-1	KCA 6.2.2/01	-
	[Thiadiazole-2- <sup>14</sup> C] FOE 5043	██████████ 1995; M-002253-01-1	KCA 6.2.2/02	-
	[Fluorophenyl-UL- <sup>14</sup> C] FOE oxalate	██████████ 1995; M-004474-01-1	KCA 6.2.2/03	-
	[1- <sup>14</sup> C] Trifluoroacetic acid	██████████ 2013; M-463376-01-1		KCA 6.2.2/04

**B 7.2.2.1.1 Poultry metabolism studies evaluated during Annex I submission and reconsidered for renewal of active substance approval**

The kinetic behaviour and the metabolism of FOE 5043 was investigated in a laying hens using two different labels, i.e. [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 (report no. MR103946) and [thiadiazole-2-<sup>14</sup>C]FOE 5043 (report no. MR106785).

<b>Reports:</b>	██████████ Metabolism of [fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in laying hens. Bayer Corporation, report no. MR103946 of 24.03.1995b
	and
	██████████, Metabolism of [thiadiazole-2- <sup>14</sup> C]FOE 5043 in laying hens, Bayer Corporation, report no.: MR106785 of 20.03.1995a
<b>Guideline:</b>	EPA § 171-4(b), Nature of Residue Livestock
<b>GLP:</b>	yes (certified laboratory)

In review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689) EFSA concluded above studies:

*“Laying hens were dosed with 5 mg/kg bw/d of fluorophenyl-U-<sup>14</sup>C and thiadiazole-2-<sup>14</sup>C labelled flufenacet, corresponding to approximately 350 times the maximum exposure of poultry. These studies demonstrate that transfer of residues to eggs and tissues is relatively low at this high dose rate. The highest residue levels were found in liver (1.4 and 10.4 mg eq./kg respectively), levels in eggs were lower (0.2 and 0.8 mg eq./kg respectively).”*

Description of these studies evaluated by RMS (France) is cited below and summarized in Table 7.2.2.1.1-1.

**Materials and methods:**

[Fluorophenyl-UL-<sup>14</sup>C]FOE 5043 with a specific radioactivity of 60.0 mCi/mMole (radiochemical purity >99%) as well as [thiadiazole-2-<sup>14</sup>C]FOE 5043 with a specific radioactivity of 18.47 mCi/mMole

(radiochemical purity >99 %) filled in gelatine capsules was administered at an oral dose level of 5 mg/kg body weight on three consecutive days in time intervals of 24 hours to ten laying hens. The hens were sacrificed 3 to 4 h after the last administration. Radioactivity was measured in the edible tissues, kidney, liver, muscle and fat at sacrifice and in the 2-day eggs. The eggs and edible tissues were analysed for parent compound and metabolites by extraction and chromatographic separation techniques followed by spectroscopic investigations.

### Findings:

[Fluorophenyl-UL-<sup>14</sup>C]FOE 5043

**Table 7.2.2.1.1-1 [Table 6.2.5a in the DAR]: Residue levels in the edible tissues and organs**

Organ	Actual residue levels (1902x) ❶	Anticipated residue levels
Liver	1.377 µg/g	<0.001µg/g
Muscle	0.201 µg/g	<0.001µg/g
Fat	0.443 µg/g	<0.001µg/g
Eggs ❷	0.153µg/g	<0.001µg/g

❶ based on a anticipated residue level of 0.041 mg/kg (see 6.4)

❷ day 3-eggs

The equivalent concentration of radioactivity in the eggs showed an increase from 0.025 µg/g obtained at 1 day after the first dosage to 0.108 µg/g after 2 days to 0.153 µg/g at sacrifice (3 days).

The metabolites identified in the edible tissues and eggs are summarised in Table 6.2.5b [current Table 7.2.2.1.1-2]. [...]

Metabolism of FOE 5043 in poultry appeared to involve the mercapturic acid pathway resulting in a wide range of methylsulfinyl and methylsulfonyl containing metabolites produced from further metabolism of the cysteine of mercapturic acid conjugates of FOE 5043. Metabolites identified in tissues included N-(4-fluorophenyl)-N-(1-methyl-ethyl)-2-(methylsulfonyl)acetamide (M7), N-(4-fluorophenyl)acetamide (M27), N-(4-fluorophenyl)-N-(2-hydroxy-1-methyl-ethyl)-2-(methylsulfonyl)acetamide (M29) (liver, muscle), N-(4-fluorophenyl)-N-(2-hydroxy-1-methyl-ethyl)-2-(methylsulfinyl)acetamide (M28) (muscle), S-[2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxoethyl]cysteine (M23) (liver), N-acetyl-S-[2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxoethyl]cysteine (M10) (liver) and N-(4-fluorophenyl)-N-(1-methylethyl acetamide (M13). Unchanged parent compound was identified in fat (55 %), in muscle (3 %) and in day-2 eggs (7 %).

Almost all of these metabolites were found in the liver except M28 and parent compound. In this organ 43 % of the residue was identified. The unextractable residues following extraction with methanol accounted for 27 % of the total radioactivity in the liver. Following 2N HCl hydrolysis of the solids, 24 % of the total radioactivity was extracted.

**Table 7.2.2.1.1-2 [Table 6.2.5b in the DAR]: Distribution of the metabolites in different organs, tissues and eggs after application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 to laying hens (values are given in % of recovered radioactivity)**

Component	% of total radioactive residue			
	Liver	Muscle	Fat	Eggs
M7	8	14	17	---
M10	9	---	---	---
M13	3	---	---	---
M23	7	---	---	---
M27	2	19	11	---
M28	---	8	---	---
M29	5	22	---	---
FOE 5043	---	3	55	7
tentatively identified	9	5	---	---
solids	27	16	6	47
Identification rate	43	71	83	7
Extraction rate	73	85	94	53

In the muscle the major degradation products identified were M29 (22 %), N-(4-fluorophenyl)acetamide (M27) representing 19% and the methylsulfonyl-containing metabolite M7 (14%). A total of 71% of the radioactivity in muscle was identified.

The major residue in fat was identified as the parent compound representing 55 % of the total radioactivity. The methylsulfonyl-containing metabolite M7 amounted to 17 %, N-(4-fluorophenyl)acetamide (M27) represented 11 %. A total of 83 % of the radioactive residue in fat was identified. Of the total radioactivity present in day-2 eggs, 7 % was tentatively identified by co-chromatography as the parent compound. In addition at least five radioactive components were detected, each ranging from 2 to 13 % of the total radioactivity. Due to the low levels of radioactive residues of these components (0.002 to 0.014 mg/kg) additional characterisation was not performed. 31% of total radioactive residue was characterised as organosoluble following acid hydrolysis.

- [Thiadiazole-2-<sup>14</sup>C]FOE 5043

Although the study with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 showed extensive metabolism of the fluorophenyl acetamide portion of the molecule, the metabolic fate of the thiadiazole portion of FOE 5043 remained undefined. To investigate the metabolism and distribution of the thiadiazole portion of the molecule [thiadiazole-2-<sup>14</sup>C]FOE 5043 was administered to laying hens (report no. MR 106785).

The residue levels at sacrifice in the edible tissues and organs were in the same order of magnitude compared to the residues seen in the [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 poultry metabolism study (Table 6.2.5c [current Table 7.2.2.1.1-3]).

**Table 7.2.2.1.1-3 [Table 6.2.5c in the DAR]: Residue levels in the edible tissues and organs**

Organ	Actual residue levels (1902x) ❶	Anticipated residue levels
Liver	10.400 µg/g	0.005 µg/g
Muscle	2.230 µg/g	0.001 µg/g
Fat	1.790 µg/g	<0.001 µg/g
Eggs ❷	0.759 µg/g	<0.001 µg/g

❶ based on a maximum anticipated residue level of 0.041 mg/kg (see 6.4)

❷ day 3-eggs

The equivalent concentration of radioactivity in the eggs showed an increase from 0.117 µg/g obtained at 1 day after the first dosage to 0.518 µg/g after 2 days to 0.759 µg/g at sacrifice (3 days).

The highest equivalent concentration was measured in liver (10.400 µg/g), followed by that obtained for muscle (2.230 µg/g) and fat (1.790 µg/g).

The metabolites identified in the edible tissues and in the eggs are summarised in Table 6.2.5d [current Table 7.2.2.1.1-4]. [...]

**Table 7.2.2.1.1-4 [Table 6.2.5d in the DAR]: Distribution of the metabolites in different organs, tissues and milk after application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 to laying hens (values are given in % of total radioactivity)**

Metabolite	% of total radioactive residue			
	Liver	Muscle	Fat	Eggs
M9	83	86	80	86
M24	9	---	---	---
FOE 5043	---	---	15	---
Identification rate	92	86	95	86
Extraction rate	92	86	97	86

The results of this study showed that FOE 5043 was rapidly cleaved at the ether bond yielding thiadone (M9). Its glucuronic acid conjugate (M24) was found in liver. Unchanged parent compound was only present in fat at a level of 15 % of the total radioactivity in fat.

In the liver 92 % of the residue was identified, 83 % being the thiadone (M9) and 9 % its glucuronic acid conjugate (M24).

In the muscle the only degradation product was identified as thiadone (M9), representing 86 % of the total radioactivity, yielding an identification rate of also 86%.

In fat the major degradation product was also identified as thiadone (M9), representing 80 % of the total radioactivity. Unchanged parent compound amounted to 15% of the total radioactive residue in fat. The identification rate was 95 %.

In the eggs the only degradation product was identified as thiadone (M9), representing 86 % of the total radioactivity, yielding an identification rate of also 86 %.

## Conclusion

**For the purpose of renewal of a.s. approval, these studies were considered as acceptable.**

<b>Report:</b>	Metabolism of [phenyl-UL- <sup>14</sup> C]FOE oxalate in laying hens, Bayer Corporation, report no.: MR106787 of 11.04.1995c
<b>Guideline:</b>	EPA § 171-4(b), Nature of Residue Livestock
<b>GLP:</b>	yes (certified laboratory)

In review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689) EFSA concluded above studies: “Since no parent compound was found in feed commodities an additional poultry metabolism study was performed using a representative metabolite (flufenacet oxalate). Laying hens were also dosed with 5 mg/kg bw/d of flufenacet oxalate, corresponding to approximately 350 times the exposure of poultry. This study also demonstrates that transfer of residues to eggs and tissues is relatively low, even at this high dose rate. The highest residue levels were found in liver (0.18 mg eq./kg), levels in eggs were lower (0.011 mg eq./kg).”

Description of this study evaluated by RMS (France) is cited below.

### Material and methods

[Fluorophenyl-UL-<sup>14</sup>C]FOE oxalate filled in gelatine capsules was administered to laying hens at an oral dose level of 5 mg/kg body weight on three consecutive days in time intervals of 24 hours. The hens were sacrificed 4 h after the last administration. Radioactivity was measured in the eggs at several sampling intervals, and in the edible tissues, liver, muscle and fat at sacrifice. The eggs and edible tissues were analysed for parent compound and metabolites by extraction and chromatographic separation techniques followed by spectroscopic investigations.

### Findings:

The residue levels at sacrifice in the edible tissues and organs are summarised in table 6.2.6a [current Table 7.2.2.1.1-5].

**Table 7.2.2.1.1-5 [Table 6.2.6a in the DAR]: Residue levels in the edible tissues and organs**

Organ	Actual residue levels (1902x) ❶	Anticipated residue levels
Liver	0.181 µg/g	<0.001 µg/g
Muscle	0.036 µg/g	<0.001 µg/g
Fat	0.045 µg/g	<0.001 µg/g
Eggs ❷	0.011 µg/g	<0.001 µg/g

❶ based on a maximum anticipated residue level of 0.041 mg/kg (see 6.4)

❷ day 3-eggs

The equivalent concentration of radioactivity in the eggs showed an increase from 0.003 µg/g obtained at 1 day after the first dosage to 0.006 µg/g after 2 days to 0.011 µg/g at sacrifice (3 days).

The highest equivalent concentration was measured in the liver (0.181 µg/g), followed by that obtained for the fat (0.045 µg/g) and muscle (0.036 µg/g).

The metabolites identified in the edible tissues and in the eggs are summarised in Table 6.2.6b [current Table 7.2.2.1.1-6].

**Table 7.2.2.1.1-6 [Table 6.2.6b in the DAR]: Distribution of the metabolites in different organs, tissues and eggs after application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 oxalate to laying hens (values are given in % of total radioactivity)**

Metabolite	% of total radioactive residue			
	Liver	Muscle	Fat	Eggs (day 3)
FOE oxalate	85	90	96	90
Identification rate	85	90	96	90
Extraction rate	93	94	98	92

The only residue found in the tissues was FOE oxalate, which accounted for 85 to 96 % of the total radioactive residue, i.e. 0.181 mg/kg in liver, 0.036 mg/kg in muscle and 0.045 mg/kg in fat. FOE oxalate also represented 90 % (0.011 mg/kg) of the total radioactive residue in the day-3 eggs.

FOE oxalate remained essentially unmetabolised in laying hens. The observed low residue levels in tissue and eggs suggested that FOE oxalate was minimally absorbed after oral administration and rapidly excreted. This was confirmed by a bioavailability study of FOE oxalate in rats (Krolski et. al. 1995), where

*the compound was also found to be unmetabolised and was excreted rapidly as unchanged compound with faeces (64 to 70 %) and urine (28 to 35 %) within 24 hours.*

## Conclusion

**For the purpose of renewal of a.s. approval, this study was considered as acceptable.**

### B 7.2.2.1.2 Additional poultry metabolism studies

Under natural, physiological and environmental conditions TFA is dissociated and appears as TFA salt. The counter cation depends on the chemical surrounding and is, thus, not defined. Therefore, TFA is expressed as the parent compound of the salts, i.e. as TFA-acid, keeping in mind that a TFA salt was administered to the animals.

Report:	KCA 6.2.2/04, [REDACTED] 2013; M-463376-01-1
Title:	[1- <sup>14</sup> C]Trifluoroacetic acid: Metabolism in the Laying Hen
Document No:	M-463376-01-1
Report No:	EnSa-12-0648, dated 2013-09-02
Guidelines and data requirements:	OECD guideline 503, Metabolism in Livestock, adopted 8-January-2007, US OPPTS guideline 860.1300, Nature of Residues – Plants, Livestock, 1996 Compliant with EU Regulation (EC) No. 1107/2009 amended by Commission Regulation (EU) No 283/2013
GLP	yes

## Executive Summary

A metabolism study with <sup>14</sup>C-labelled Na-TFA was conducted with six laying hens as TFA revealed to be a major metabolite in plants that were treated with flufenacet and are intended as poultry feed. This study is needed for a dietary risk assessment including food of animal origin to address the transfer of TFA residue from feedstuffs to eggs and edible animal tissue.

TFA was orally administered per gavage as <sup>14</sup>C-TFA-Na to the hens for 14 consecutive days with one dose per day. The dose level expressed as trifluoroacetic acid, TFA, was 0.50 mg/kg bw/day corresponding to 7.84 mg TFA/kg dry feed/day.

The radioactive residues in eggs reached a mean residue plateau of 0.391 mg TFA parent equivalents/kg (mg equ/kg) after 7 daily administrations. The birds were slaughtered six hours after the last dose and the radioactive residues were determined in edible organs and tissues. Average residues from six birds accounted for 0.090 mg equ/kg in fat, 0.615 mg equ/kg in muscle, 0.760 mg equ/kg in liver, 1.343 mg equ/kg in kidneys, and 1.101 mg equ/kg in skin.

The samples were extracted with acetonitrile/water and the extracts analysed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic <sup>14</sup>C-TFA reference and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. Therefore, the conclusion has to be drawn that the total radioactivity in eggs, organs and tissues consisted of the unchanged TFA.

By comparison of the residue levels in feed, eggs, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: eggs (at plateau): 0.0499; muscle: 0.0784; fat: 0.0115; liver: 0.0969.

## Material and methods

### Test Material

Structural formula	<p>* denotes the <math>^{14}\text{C}</math> label</p>
Chemical name	Sodium trifluoroacetate
CAS RN	2932-18-4
Empirical formula	$\text{C}_2\text{F}_3\text{NaO}_2$
Company code	BCS-AZ56567
Molar mass (non-labelled)	136.01 g/mol
Label	1- $^{14}\text{C}$
Specific radioactivity	4.08 MBq/mg = 110.14 $\mu\text{Ci/mg}$
Radiochemical purity	>98% by TLC and HPLC (radio-detection)
Remark	<p>Trifluoroacetate appeared as anion under physiological and environmental conditions. The corresponding cation depends on the chemical surrounding and, thus, is not defined. Therefore, the residue levels of trifluoroacetate are expressed as the parent substance trifluoro-acetic acid (TFA). A conversion is conducted via the ratio of the molar masses:</p> <p><math>\text{MM (trifluoroacetic acid)} / \text{MM (sodium trifluoro acetate)} = 114.02/136.01 = 0.8383</math></p> <p>The specific radioactivity of the respective trifluoroacetic acid (TFA) is therefore: <math>4.08 \text{ MBq/mg} / 0.8383 = 4.87 \text{ MBq/mg}</math></p>

### Test Animal

Species	Hen ( <i>Gallus gallus domesticus</i> )
Breed	White Leghorn
Sex, number	Six female laying hen
Mean body weight	1.57 kg at test start (1.45 – 1.65 kg)
Age	Approx. 6 months
Acclimatization	14 days before administration
Housing	Each 1 bird per stainless steel metabolism cage, approx. 24°C, approx. 31% rel. humidity, 16/8 hours light/dark cycle, 10-15 air changes per hour
Identification	Individual animal number using cage cards and wing tags
Feed and water	Commercial hen feed supplemented by eggshells and crushed marine shells, <i>ad libitum</i> Tap water from local supplier, <i>ad libitum</i>
Health status	Acceptable according to veterinary investigation

### Preparation of the dosing mixtures and administration

The radiolabelled solid sodium trifluoroacetate was dissolved in water resulting in a concentration of 0.59 mg/mL (corresponding to 0.49 mg TFA/mL). The exact concentration, radiochemical purity and the identity were determined by radioassaying, radio-TLC and LC-MS/MS using small aliquots of the dosing solution. Dosing aliquots of 1.0 mL/kg bw were orally administered by gavage using a syringe attached to an animal-feeding knob cannula. Directly after dosage, the swallowing reflex was supported by a gentle massage of the throat in direction of the crop. Each bird received one dose per day for 14 consecutive days. The average daily dose was 0.79 mg TFA per bird corresponding to 0.50 mg TFA/kg bw/day. With reference to the daily feed consumption this dose corresponded to 7.84 mg TFA/kg dry feed/day. This dose was tolerated without any observable toxicological effects.

### Collection and processing of eggs and excreta

During the test, the grates of the cages were inspected for egg production once daily and the number of eggs was recorded for all hens. The eggs were collected during the 24 hour period after each administration and labelled with animal number and date. After removal of the shells, the contents of each egg were

weighed and thoroughly mixed afterwards. An aliquot of each homogenate radioassayed and the remaining samples were stored in a freezer until metabolite analysis.

The excreta of each hen were collected from the collecting tins as far as possible quantitatively in daily intervals until sacrifice. The individual samples were homogenized after adding of water, before the total weights were recorded. An aliquot of each fraction was radioassayed and the remaining samples were stored in a freezer until metabolite analysis.

#### Sacrifice and collection of organs and tissues

The animals were sacrificed approx. 6 hours after the last dose. Each hen was transferred into a special cage, weighed and anaesthetized using carbon dioxide gas. Under general anaesthesia the animals were sacrificed by decapitation followed by exsanguination. The following organs and tissues were dissected: muscle (leg and thorax), fat (subcutaneous), liver (without gall bladder), skin (without subcutaneous fat) kidney and eggs from the ovary as well as oviduct.

The tissue samples were weighed and passed several times through a mincing machine in half-frozen state. The resulting homogeneous pulp was radioassayed and stored frozen ( $\leq -18^{\circ}\text{C}$ ) until metabolite analysis.

#### Radioassaying and processing of samples

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed  $^{14}\text{CO}_2$  was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC. The limit of quantification (LOQ) of radioassaying depended on the specific radioactivity of the test substance, the amount of aliquot measured and the background radioactivity. It was exemplarily given as 0.0005 mg equ/kg.

For metabolism investigations, aliquot samples from eggs, muscle and liver were conventionally extracted with acetonitrile/water (8/2; v/v, 3x) and pure acetonitrile using a high-speed stirrer. Fat was extracted with n-heptane/acetonitrile (9/1; v/v) and acetonitrile/water/n-heptane (7/2/1; v/v/v). The liquid phases were filtrated from the solids. In case of fat, the extracts were separated in an unpolar (n-heptane) and a polar (acetonitrile/water) fraction. The unpolar fraction was again extracted with acetonitrile/water and the polar fraction with n-heptane. The total radioactivity extracted from fat finally partitioned into the combined polar acetonitrile/water phase. The acetonitrile/water extracts were concentrated and analyzed by radio-HPLC and radio-TLC. The remaining solids were radioassayed via combustion.

#### Radio-chromatography and mass spectrometry of samples

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 5  $\mu\text{m}$  particles) that was operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at  $40^{\circ}\text{C}$ . The system was equipped with an UV detector (254 nm) and a radiomonitor with a solid glass scintillator (cell volume 370  $\mu\text{L}$ ). The LOQ was derived from background level of the baseline and the highest peak in the chromatogram. It ranged from 0.001 mg equ/kg (muscle and fat extract) to 0.004 mg equ/kg (egg extract). Column recovery was determined by comparison of the eluted radioactivity with column and detector and without column and detector. It generally accounted for 99.7%.  $^{14}\text{C}$ -labelled TFA was co-injected to identify the residues in the samples.

Radio-TLC was conducted on a silica gel TLC plate (20 x 20 cm) that was developed with a solvent mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/22/1, v/v/v/v). Following development the radioactive spots were detected by radioluminography via exposure of an imaging plate for 14 hours. The detection limit was approximately 5-10 dpm/spot after an exposure period of at least 14 hours.  $^{14}\text{C}$ -TFA was also used as reference standard.

The test substance TFA was identified by LC-MS/MS consisting of anion exchange chromatography and a high resolution mass spectrometer. For chromatography an anion exchanging Dionex column was eluted with an aqueous solution of 20 mmol KOH as isocratic liquid phase. A Q-Exactive mass spectrometer was operated in the mode of electro-spray ionization.

## Findings

### Recovery of radioactivity in eggs, excreta and analyzed organs and tissues

Six hours after the last of 14 oral doses of  $^{14}\text{C}$ -labelled TFA at a dose rate of 0.50 mg/kg bw/day 94.97% of the total radioactivity was recovered in eggs, excreta, muscle, fat, liver and kidney. The remaining 5% of the total dose were assumed to be associated with the gastro-intestinal tract and the remaining body.

88.01% of the total dose was detected in the excreta. 1.91% of the total dose were found in the eggs and 5.06% were detected in the dissected edible organs and tissues with approx. 70% of this radioactivity (3.53% of dose) being associated with the skeletal muscle (assuming 40% of the body weight for skeletal muscle).

### Radioactive residues in the eggs

The total radioactive residues (TRR) in the eggs ranged from 0.123 mg equ/kg at day two to 0.408 mg equ/kg at day 13. The time course of the TRR showed a more or less linear increase until seven administrations at a dose rate of 7.84 mg TFA/kg dry feed/day. By the eighth administration, TRR reached a pronounced residue plateau. The weighted mean amounted to 0.391 mg equ/kg between the 7<sup>th</sup> and 13<sup>th</sup> day (8<sup>th</sup> – 14<sup>th</sup> administration). The residue level of the last egg sample (0.607 mg equ/kg) was excluded from plateau calculation since the interval between dosing and egg collection (0.25 day) was significantly shorter than at the other days. Daily TRR levels in the eggs are compiled in Table 7.2.2.1.2-1.

### Radioactive residues in dissected organs and tissues

The TRR in edible organs and tissues ranged from fat amounting to 0.090 mg equ/kg to kidney amounting to 1.343 mg equ/kg. Skeletal muscle accounted for 0.615 mg equ/kg and skin for 1.101 mg equ/kg. The residue levels in all edible tissues of hen are compiled in Table 7.2.2.1.2-2.

### Extraction efficiency and identification of extracted residues

The majority of the radioactive residues (99.9% - 100% of TRR) in eggs, muscle, liver and excreta (Day 13) was extractable with acetonitrile/water (8/2; v/v) and pure acetonitrile. From fat, 95% of TRR could be extracted with heptane and acetonitrile/water that completely partitioned into the polar phase. Negligible amounts of  $\leq 0.1\%$  of the TRR ( $\leq 0.001$  mg/kg) remained unextractable. Following concentration, 99.5% to 100% of the TRR in the extracts were analysed and quantified by radio-HPLC and radio-TLC.

The radio-chromatographic profiles of all extracts (eggs, muscle, liver, kidney, fat, and excreta) showed only one polar radioactive peak. Co-chromatography with the reference standard  $^{14}\text{C}$ -TFA resulted in the same single peak that was unambiguously identified as radiolabelled TFA since two different chromatographic systems (reversed phase HPLC and straight phase TLC) were used. No other peak could be observed. Therefore, the total radioactivity in all samples represented unchanged TFA. Thus, the rate of identification in the samples was excellent amounting to 99.5 – 100% of TRR in all extracts.

### Transfer factors of residue transfer of TFA from animal fodder to food of animal origin

The TFA transfer factors (TF) were calculated as mean ratio between the radioactive residues in animal fodder (based on dry mass) and the total radioactive residues in eggs, and edible organs and tissues of the six hens. Any correction for metabolic conversion products of TFA is not needed as total radioactive residue was represented by the administered test substance (see before). These transfer factors ranging from 0.0115 (fat) to 0.1713 (kidney) are listed in detail in Table 7.2.2.1.2-3.

## Conclusion

Following repeated oral administration of  $^{14}\text{C}$ -labelled sodium trifluoroacetate (TFA-Na) to six laying hens for 14 consecutive days at a dose level of 0.50 mg TFA/kg bw/day (corresponding to 7.84 mg TFA/kg dry feed/day) the radioactive residues in eggs reached a plateau level of 0.391 mg equ/kg after 7 daily administrations. 14 days after the first administration the hens were slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 1.101 mg equ/kg in fat, 0.615 mg equ/kg in muscle, 0.760 mg equ/kg in liver and 1.343 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analysed by radio-HPLC (reversed phase) and radio-TLC (straight

phase). Identification of the radioactivity in all samples using co-chromatography with authentic  $^{14}\text{C}$ -TFA and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. As a conclusion it can be stated that TFA is metabolically stable in poultry. It was rapidly excreted as not more than 5% of the total dose was detected in organs and tissues 6 hours after administration of the last dose.

By comparison of the residue levels in feed, eggs, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: eggs (at plateau): 0.0499; muscle: 0.0784; fat: 0.0115; liver: 0.0969; kidney: 0.1713.

**Table 7.2.2.1.2-1: Total radioactive residues (TRR) in eggs of hens orally administered with  $^{14}\text{C}$ -TFA at a dose of 7.84 mg TFA/kg dry feed/day for 14 consecutive days (mean of 6 hens)**

Time after the 1 <sup>st</sup> administration	No. of administration	TRR in freshly laid eggs	Remark
[days]		[mg equ/kg]	
0	1	no egg sampled	
1	2	no egg sampled	
2	3	0.123	
3	4	0.218	Continuous increase of residue level
4	5	0.262	
5	6	0.310	
6	7	0.362	
7	8	0.396	
8	9	0.406	Plateau level of residues
9	10	0.410	
10	11	0.402	
11	12	0.395	
12	13	0.405	
13	14	0.408	
13.25	---	0.607	Short collection period
Weighted mean plateau level of 8 <sup>th</sup> – 14 <sup>th</sup> administration (days 7-13)		0.391	

**Table 7.2.2.1.2-2: Radioactive residues in organs and tissues of hens 6 hours after the last of 14 doses of  $^{14}\text{C}$ -TFA at a dose level of 7.84 mg TFA/kg dry feed/day (mean of 6 hens)**

Organ/Tissue	Mean Residue Level [mg equ/kg]
Liver	0.760
Kidney	1.343
Skeletal muscle, total	0.615
Leg muscle	0.712
Thorax muscle	0.507
Skin without fat	1.101
Subcutaneous fat	0.090
Eggs from ovary/oviduct	0.754

**Table 7.2.2.1.2-3: Transfer factors for residue transfer of  $^{14}\text{C}$ -TFA from animal feed to eggs, muscle, fat, liver and kidney of hens following repeated administration at a dose level of 7.84 mg TFA/kg dry feed/day**

Milk/Organ/Tissue	Residue level [mg equ/kg]	Transfer factor (TF)
Eggs (at residue plateau)	0.391	0.0499
Muscle	0.615	0.0784
Fat	0.090	0.0115
Liver	0.760	0.0969
Kidney	1.343	0.1713
Skin	1.101	0.1404

### B 7.2.2.2 Lactating ruminants

An overview on the metabolism studies of flufenacet and major plant metabolites on lactating goat is presented in Table 7.2.2.2-1.

**Table 7.2.2.2-1: Overview of goat metabolism studies with  $^{14}\text{C}$ -label flufenacet**

Animal	Label	Report	Submission	
			EU baseline dossier, Annex II , Section 4, Point 6	Presented in supplementary dossier Section 6
Lactating goat	[Fluorophenyl- $^{14}\text{C}$ ] FOE 5043	██████████ 1995; M-002250-01-1	KCA 6.2.3/01	-
	[Thiadiazole-2- $^{14}\text{C}$ ] FOE 5043	██████████ 1995; M-002248-01-1	KCA 6.2.3/02	-
	[Fluorophenyl- $^{14}\text{C}$ ] FOE oxalate	██████████ 1995; M-004478-01-1	KCA 6.2.3/03	-
	[Thiadiazole-2- $^{14}\text{C}$ ] thiadone-N-glucoside	██████████ 2002; M-079251-01-1	-	KCA 6.2.3/04
	[1- $^{14}\text{C}$ ] Trifluoroacetic acid	██████████ 2013; M-444459-01-1	-	KCA 6.2.3/05

#### B 7.2.2.2.1 Goat metabolism studies evaluated during Annex I submission and reconsidered for renewal of active substance approval.

The kinetic behaviour and the metabolism of FOE 5043 was investigated in a lactating goat using two different labels, i.e. [fluorophenyl- $^{14}\text{C}$ ]FOE 5043 (report no. MR105184) and [thiadiazole-2- $^{14}\text{C}$ ]FOE 5043 (report no. MR106784).

<b>Reports:</b>	Metabolism of [fluorophenyl-UL- <sup>14</sup> C]FOE 5043 in lactating goat, Bayer Corporation, report no.: MR105184 of 03.03.1995a
	and
	Metabolism of [thiadiazole-2- <sup>14</sup> C]FOE 5043 in a lactating goat, Bayer Corporation, report no.: MR106784 of 11.07.1995
<b>Guideline:</b>	EPA § 171-4(b), Nature of Residue Livestock
<b>GLP:</b>	yes (certified laboratory)

Description of these studies evaluated by RMS (France) is cited below.

#### **Materials and methods:**

[Fluorophenyl-UL-<sup>14</sup>C]FOE 5043 with a specific radioactivity of 60.0 mCi/mMole (radiochemical purity >99 %) as well as [thiadiazole-2-<sup>14</sup>C]FOE 5043 with a specific radioactivity of 18.5 mCi/mMole (radiochemical purity 100 %) filled in gelatine capsules was administered to one lactating goat for each experiment (body weight 46 kg and 49 kg) at an oral dose level of 5 mg/kg body weight on three consecutive days in time intervals of 24 hours. The goat was sacrificed 4 h after the last administration. Radioactivity was measured in milk at several sampling intervals, and in the edible tissues, kidney, liver, muscle and fat at sacrifice. The milk and edible tissues were analysed for parent compound and metabolites by extraction and chromatographic separation techniques followed by spectroscopic investigations.

#### **Findings:**

- [Fluorophenyl-UL-<sup>14</sup>C]FOE 5043

Due to the fast elimination kinetics, the resultant residue levels in most tissues and organs as well as in milk were not elevated in relation to the dose level except those determined in the liver and kidney representing the main metabolising and the main excretory organ, respectively. The residue levels at sacrifice in the edible tissues and organs are shown in Table 6.2.3a [current Table 7.2.2.2.1-1].

**Table 7.2.2.2.1-1 [Table 6.2.3a in the DAR]: Residue levels in the edible tissues and organs**

Organ	Actual residue levels (668x)❶	Anticipated residue levels
Liver	3.726 µg/g	0.006 µg/g
Kidney	3.774 µg/g	0.006 µg/g
Muscle	0.264 µg/g	<0.001 µg/g
Fat	0.276 µg/g	<0.001 µg/g
Milk ❷	0.302 µg/ml	<0.001 µg/ml

❶ based on a maximum anticipated residue level of

0.25 mg/kg (see 6.4)

❷ (maximum concentration at sacrifice)

The equivalent concentration of radioactivity in the milk showed an increase from 0.148 µg/ml obtained at 1 day after the first dosage to 0.222 µg/ml after 2 days to 0.302 µg/ml at sacrifice (3 days). Milk was collected twice daily, just before dosing and in the evening. The milk collected in the evening was composited with the morning milk collected before the next dose.

The highest equivalent concentration was measured in the kidney (3.774 µg/g), followed by that obtained for the liver (3.726 µg/g). This result reflects the significance of these organs for excretion and metabolism of the compound. The concentrations in kidney and liver were followed in decreasing order by those obtained for fat (0.276 µg/g), and muscle (0.264 µg/g), respectively.

The metabolites identified in the edible tissues and in the milk are summarised in Table 6.2.3b [current Table 7.2.2.2.1-2].

**Table 7.2.2.1-2 [Table 6.2.3b in the DAR]: Distribution of the metabolites in different organs, tissues and milk after application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 to a dairy goat (values are given in % of total radioactivity)**

Metabolite	% of total radioactive residue				
	Kidney	Liver	Muscle	Fat	Milk
M7	1	7	16	15	14
M10	24	--	--	--	--
M15	3	3	22	17	20
M22	--	58	--	--	--
M23	53	16	41	55	53
FOE 5043	--	--	2	2	--
Identification rate	81	84	81	89	87
Extraction rate	96	95	86	93	93

FOE 5043 is extensively metabolised in the goat. The first metabolism step is conjugation with glutathione. Further biodegradation follows the mercapturic acid pathway, with additional formation of cysteine- or mercapturic acid conjugates. Major metabolites in tissues included N-(4-fluorophenyl)-N-isopropyl-2-(S-glutathionyl) acetamide (M22), S-[2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxoethyl]cysteine (M23), N-acetyl-S-[2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxoethyl]cysteine (M10), N-(4-fluorophenyl)-N-(1-methyl-ethyl)-2-(methylsulfonyl)acetamide (M7) and 4-fluoroaniline methylsulfonyl acetamide (M15). Unchanged parent compound was identified in small amounts (2 %) in fat and muscle.

Almost all of these metabolites were found in the liver. In this organ 84 % of the residue was identified, 58 % is attributable to the glutathione conjugate (M22). 16% was identified as the cysteine conjugate (M23). The methylsulfonyl-containing metabolites M7 and M15 amounted to 7 and 3 %, respectively. No parent compound was detected in the liver demonstrating the high metabolism rate.

In the kidney a total of 81 % of the radioactivity was identified. The major metabolite in kidney was the cysteine conjugate (M23), representing 53 % of the total radioactivity. The corresponding N-acetyl conjugate (M10) amounted to 24%. The methylsulfonyl-containing metabolites M7 and M15 amounted to 1 and 3 %, respectively.

In the muscle the major degradation product identified was the cysteine conjugate (M23), representing 49 % of the total radioactivity. The methylsulfonyl-containing metabolites M7 and M15 amounted to 16 and 22%, respectively. Parent compound was detected in a small amount of 2 %. A total of 89 % of the radioactivity in muscle was identified.

The major degradation product in fat was identified as the cysteine conjugate (M23), representing 55 % of the total radioactivity. The methylsulfonyl-containing metabolites M7 and M15 amounted to 15 and 17%, respectively. Parent compound was detected in a small amount of 2 %, yielding an identification rate of 89 %.

The metabolites identified in the milk represented 87 % of the total residue. The major degradation product was again as in kidney, muscle and fat the cysteine conjugate (M23), representing 53 % of the total radioactivity. The methylsulfonyl-containing metabolites M7 and M15 amounted to 14 and 20 %, respectively.

- [Thiadiazole-2-<sup>14</sup>C]FOE 5043

Although the study with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 showed extensive metabolism of the fluorophenyl acetamide portion of the molecule, the metabolic fate of the thiadiazole portion of FOE 5043

remained undefined. To investigate the metabolism and distribution of the thiadiazole portion of the molecule [thiadiazole-2-<sup>14</sup>C]FOE 5043 was administered to a lactating goat (report no. MR106784).

The residue levels at sacrifice in the edible tissues and organs in the [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 goat metabolism study are given in table 6.2.3c [current Table 7.2.2.1-3].

**Table 7.2.2.1-3 [Table 6.2.3c in the DAR]: Residue levels in the edible tissues and organs**

Organ	Actual residue levels (668x) ❶	Anticipated residue levels
Liver	16.956 µg/g	0.025 µg/g
Kidney	20.409 µg/g	0.030 µg/g
Muscle	3.816 µg/g	0.006 µg/g
Fat	2.846 µg/g	0.004 µg/g
Milk ❷	0.816 µg/ml	0.001 µg/ml

❶ based on a maximum anticipated residue level of

0.25 mg/kg (see 6.4)

❷ (maximum concentration at sacrifice)

The equivalent concentration of radioactivity in the milk showed an increase from 0.258 µg/ml obtained at 1 day after the first dosage to 0.585 µg/ml after 2 days to 0.816 µg/ml at sacrifice (3 days). Milk was collected twice daily, just before dosing and in the evening. The milk collected in the evening was composited with the morning milk collected before the next dose.

The highest equivalent concentration was measured in the kidney (20.409 µg/g), followed by that obtained for the liver (16.956 µg/g). The concentrations in kidney and liver were followed in decreasing order by those obtained for muscle (3.816 µg/g), and fat (2.846 µg/g), respectively.

The metabolites identified in the edible tissues and in the milk are summarised in Table 6.2.3d [current Table 7.2.2.1-4]. [...]

**Table 7.2.2.1-4 [Table 6.2.3d in the DAR]: Distribution of the metabolites in different organs, tissues and milk after application of [thiadiazole-2-<sup>14</sup>C]FOE 5043 to a dairy goat (values are given in % of total radioactivity)**

Metabolite	% of total radioactive residue				
	Kidney	Liver	Muscle	Fat	Milk
M24	9	5	---	---	8
M9	89	86	84	89	45
Unknown metabolites	1	2	1	---	32
Identification rate	98	91	84	89	53
Extraction rate	99	95	96	97	87

The results of this study showed that FOE 5043 was rapidly cleaved at the ether bond yielding thiadone (M9) which was primarily conjugated to glucuronic acid (M24) prior to elimination in the excreta. Unchanged parent compound was not present in any tissue, organ or milk.

Three metabolites were found in the liver. In this organ 91 % of the residue was identified, 86% was attributable to the thiadone (M9), 5 % to the glucuronic acid conjugate (M24) and 2 % to an unknown metabolite.

*In the kidney a total of 98 % of the radioactivity was identified. The major metabolite in kidney was the thiadone (M9) with 89 %. The glucuronic acid conjugate (M24) amounted to 9 % and an unknown metabolite to 1 %.*

*In the muscle the major degradation product was identified as thiadone (M9), representing 84 % of the total radioactivity. An unknown metabolite amounted to 1 %. The identification rate was 84 %.*

*The only degradation product in fat was identified as thiadone (M9), representing 89 % of the total radioactivity, yielding an identification rate of also 89 %.*

*The metabolites identified in the milk represented 85 % of the total residue. The major degradation product was again the thiadone (M9), representing 45 % of the total radioactivity. The glucuronic acid conjugate (M24) amounted to 8 % and an unknown metabolite to 32 %. All efforts to identify this unknown metabolite in the milk extracts failed. Acidic hydrolysis due to the polar behaviour of the metabolite resulted in a loss of more than 90 % of the radioactivity suggesting the unknown to be a conjugate or derivative of thiadone because thiadone is highly volatile.*

*The residue levels after administration of [thiadiazole-2-<sup>14</sup>C]FOE 5043 were 3 to 14 times higher as compared to those obtained in the study with [fluorophenyl-UL-<sup>14</sup>C]FOE 5043. The initial step in the metabolism of FOE 5043 in goat is the cleavage of the ether bond, resulting in two moieties: Fluorophenyl and thiadone, which is immediately conjugated with glucuronic acid. The two different compounds undergo further metabolism and elimination at different rates depending on the polarity of the metabolites. Generally, unless a compound is biotransformed to more polar metabolites that are ultimately excreted, the compound will remain in the animal, mostly in the fatty tissues, for longer periods of time. Since more than 80 % of the tissue residues from the [thiadiazole-2-<sup>14</sup>C]FOE 5043 study was present as free thiadone and not as the more polar conjugate, elimination of thiadone is expected to be slower. Comparatively, since the residues from the [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 experiment were mostly polar metabolites, elimination of these compounds from the tissues is probably faster than the elimination from the thiadiazole portion of FOE 5043 resulting in lower residue levels. [...]*

## Conclusion

**For the purpose of renewal of a.s. approval, these studies were considered as acceptable.**

Due to the fact that no parent compound was found as residue in feed items, an additional goat metabolism study was performed using one representative plant residue, i.e. FOE oxalate to determine the metabolic fate of this representative plant residue in ruminants.

<b>Report:</b>	Metabolism of [phenyl-UL- <sup>14</sup> C]FOE oxalate in a lactating goat, Bayer Corporation, report no.: MR106786 of 31.03.1995b
<b>Guideline:</b>	EPA § 171-4(b), Nature of Residue Livestock
<b>GLP:</b>	yes (certified laboratory)

Description of this study evaluated by RMS (France) is cited below.

## Material and methods:

*[Fluorophenyl-UL-<sup>14</sup>C]FOE oxalate with a specific radioactivity of 50.2 mCi/mMole (radiochemical purity 98 %) filled in gelatine capsules was administered to one lactating goat (body weight 41 kg) at an oral dose level of 5.12 mg/kg body weight on three consecutive days in time intervals of 24 hours. The goat was sacrificed 4 h after the last administration. Radioactivity was measured in milk at several sampling intervals, and in the edible tissues, kidney, liver, muscle and fat at sacrifice. The milk and edible tissues were analysed for parent compound and metabolites by extraction and chromatographic separation techniques followed by spectroscopic investigations.*

**Findings:**

The residue levels at sacrifice in the edible tissues and organs are summarised in table 6.2.4a [current Table 7.2.2.2.1-5].

**Table 7.2.2.2.1-5 [Table 6.2.4a in the DAR]: Residue levels in the edible tissues and organs**

Organ	Actual residue levels (684x) ❶	Anticipated residue levels
Liver	0.230 µg/g	<0.001 µg/g
Kidney	1.204 µg/g	0.002 µg/g
Muscle	0.044 µg/g	<0.001 µg/g
Fat	0.036 µg/g	<0.001 µg/g
Milk ❷	0.017 µg/ml	<0.001 µg/ml

❶ based on a maximum anticipated residue level of 0.25 mg/kg (see 6.4)

❷ (maximum concentration at sacrifice)

The equivalent concentration of radioactivity in the milk showed an increase from 0.008 µg/ml obtained at 1 day after the first dosage to 0.011 µg/ml after 2 days to 0.017 µg/ml at sacrifice (3 days). Milk collected during the 24 hours following the first and second doses were considered the day-1 and day-2 milk samples. Milk collected after the third dose and immediately before sacrifice was considered the day-3 milk sample.

The highest equivalent concentration was measured in the kidney (1.204 µg/g), followed by that obtained for the liver (0.230 µg/g). The concentrations in muscle (0.044 µg/g) and fat (0.036 µg/g) were significantly lower.

The metabolites identified in the edible tissues and in the milk are summarised in Table 6.2.4b [current Table 7.2.2.2.1-6].

**Table 7.2.2.2.1-6 [Table 6.2.4b in the DAR]: Distribution of the metabolites in different organs, tissues and milk after application of [fluorophenyl-UL-<sup>14</sup>C]FOE 5043 oxalate to a dairy goat (values are given in % of recovered radioactivity)**

Metabolite	% of total radioactive residue				
	Kidney	Liver	Muscle	Fat	Milk (day 2)
FOE oxalate	99	77	94	86	38
Solids	1	2	2	2	32
Unknown 1	---	---	---	---	5
Unknown 2	---	---	---	---	8
Losses during clean up	---	21	---	12	17
Identification rate	99	77	94	86	38
Extraction rate	99	98	98	98	68

The only residue found in the tissues was FOE oxalate, which accounted for 77 to 99 % of the total radioactive residue, i.e. 1.192 mg/kg in kidney, 0.177 mg/kg in liver, 0.041 mg/kg in muscle and 0.031 mg/kg in fat. FOE oxalate also represented 38 % (0.004 mg/kg) of the total radioactive residue in the day-2 milk sample. Two minor unidentified metabolites representing 5 to 8 % (0.001 mg/kg) were also detected.

FOE oxalate was essentially unmetabolised by the lactating goat. The observed low residue levels in tissue and milk suggested that FOE oxalate was minimally absorbed after oral administration and rapidly excreted. This was confirmed by a bioavailability study of FOE oxalate in rats (Krolski et. al. 1995), where

*the compound was also found to be unmetabolised and was excreted rapidly as unchanged compound with faeces (64 to 70 %) and urine (28 to 35 %) within 24 hours.*

## Conclusion

**For the purpose of renewal of a.s. approval, this study was considered as acceptable.**

### B 7.2.2.2.2 Additional goat metabolism studies

In metabolism studies of [thiadiazole-2-<sup>14</sup>C]flufenacet in soybeans and rotational crops (e.g. wheat) a major residue component in ruminant feed (forage, hay, straw) was detected as thiadone-*N*-glucoside (M25, THNG), whereas the parent substance was not present. Therefore, a metabolism study with a lactating goat was conducted using this metabolite to discover the residues in food of animal origin. This study was performed on request of the US EPA to investigate the metabolic fate and bioavailability of THNG in a lactating ruminant.

<b>Report:</b>	KCA 6.2.3/04, [REDACTED] 2002; M-079251-01-1
<b>Title:</b>	The metabolism of FOE 5043 Thiadone <i>N</i> -Glycoside in a Lactating Goat.
<b>Document No:</b>	M-079251-01-1
<b>Report No:</b>	F3041002
<b>Guidelines and data requirements</b>	EPA Ref.: 860.1300 – Nature of residue – livestock; 870.7485 – Metabolism and pharmacokinetics
<b>GLP</b>	yes

## Executive Summary

To determine the metabolic fate of thiadone-*N*-glucoside (THNG, M25) in ruminants [thiadiazole-2-<sup>14</sup>C]THNG was administered orally as a single dose to a lactating pygmy goat at a dose rate of 0.432 mg/kg bw. corresponding to 16.3 mg/kg feed.

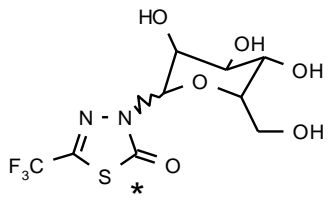
[Thiadiazole-2-<sup>14</sup>C]THNG was well absorbed and metabolized. Recovered radioactivity accounted to 91% of the dose. The majority of radioactivity was (72% of the dose) was excreted with the urine, and a smaller amount was excreted with the faeces (7% of the dose). Very little of the dose (< 1%) was observed in the milk. The maximum residue level in milk (0.040 mg THNG equ/kg) was detected in the milk secreted at the day of administration.

Residue levels found in tissues were 0.215 mg equ/kg in whole blood, 0.175 mg equ/kg in kidneys, 0.125 mg equ/kg in the liver, 0.059 mg equ/kg in the GIT, 0.025 mg equ/kg in muscle tissue, and 0.008 – 0.040 mg equ/kg in milk sampled until day 7 after administration.

The metabolism of THNG was through oxidative and hydrolytic processes and conjugation as concluded from the metabolites excreted with the urine. The main residue in liver, kidney, muscle and fat was free thiadone. However, thiadone is expected to be negligible in food of animal origin as the goat in this study was significantly overdosed with THNG. In milk, no thiadone was detectable. From the metabolites found in the urine a proposed metabolic pathway was concluded. It is shown in Figure 7.2.2.2.2-1.

## Material and methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	Thiadone-N-glucoside
Empirical formula	C <sub>9</sub> H <sub>11</sub> F <sub>3</sub> N <sub>2</sub> O <sub>6</sub> S
Company code	THNG
Molar mass (non-labelled)	332.26 g/mol
IUPAC name	3-hexopyranosyl-5-(trifluoromethyl)-2,3-dihydro-1,3,4-thiadiazol-2-one; 3-glucosyl-5-trifluoromethyl-1,3,4-thiadiazol-2(3 <i>H</i> )-one
Label	[thiadiazole-2- <sup>14</sup> C]
Specific radioactivity	9.41 mCi/mmol = 63000 dpm/μg (1.048 MBq/mg)
Radiochemical purity	>99% by HPLC (radio-detection)

### Test Animal

Species	Pygmy goat
<i>In-vivo</i> phase	Southwest Biolabs, Inc.; Las Cruces, NM, USA
Analytical phase	Bayer Research Park, Stilwell, KS, USA
Sex, number	One female lactating goat
Body weight	18.6 kg at receiving
Age	Approx. 2.5 years
Acclimatization	Two days before administration
Housing	Stainless steel metabolism cage, 19-31°C, 20-79% rel. humidity, 14/10 hours light/dark cycle
Feed and water	Ruminant feed, alfalfa pellets, hay, <i>ad libitum</i> Fresh potable water, <i>ad libitum</i>
Health status	Normal and acceptable according to veterinary investigation

### Preparation of the dosing mixtures and administration

The solid radiolabelled test substance was dissolved in a small amount of methanol and filled into a gelatin capsule that contained α-lactose. The methanol was allowed to evaporate, and the capsule was sealed at ambient temperature. The sealed capsule was orally administered using a balling gun. The actual dose rate was 0.432 mg/kg bw/day corresponding to 16.3 mg/kg in feed based on an average feed consumption of 0.493 kg feed/day.

### Collection of milk, urine and faeces

The goat was milked twice daily in the morning and evening until 168 hours post dose. The milk samples were weighed, subsampled and stored frozen.

Urine and faeces were separately collected on a daily basis until 168 hours post dose (additional urine collection: 6 and 12 hours after dosing). Faeces samples were blended with distilled water until homogenous. Aliquots of the milk and excreta samples were radioassayed.

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Sacrifice and collection of organs and tissues

A major portion of blood was collected prior to termination of the animal. On day 7, the goat was humanly terminated with a captive bolt pistol. At necropsy, bile, liver, kidneys, fat, muscle, the GI tract and the residual carcass were collected, weighed and stored frozen. Liver, kidney and fat samples were homogenized with dry ice.

All samples were shipped to the Bayer Research Park in frozen stage for analysis.

Sample extraction and processing

Urine profiles were determined by radio-HPLC for each collection point. The identification and characterization of the radioactive compounds were made for a composite urine sample from all time points.

Faeces samples were extracted four times with acetonitrile/water (9/1, v/v). The combined extracts were radioassayed and purified by passing through a C18 solid phase extraction cartridge, concentrated. The solids were further extracted with methanol, 1N aqueous hydrochloric acid and 2N sodium hydroxide in succeeding steps, each time for 18 hours under reflux. Each subsample was radioassayed.

Day-1 milk samples were lyophilized and the resulting solid extracted three times with methanol followed by extraction with water/acetonitrile (9/1, v/v). The methanol extract was radioassayed and analyzed by radio-HPLC.

Blood samples were mixed with acetonitrile and the resulting suspension separated by centrifugation. The supernatant was evaporated to dryness and dissolved in water/acetonitrile (9/1, v/v) for radioassaying and analysis by radio-HPLC.

Homogenized liver, kidney and muscle samples were separately extracted three times with acetonitrile/water (9/1, v/v). The combined extracts were concentrated to dryness and redissolved in acetonitrile. In case of liver, the resulting acetonitrile solution was partitioned against n-hexane (3x). In each case, the acetonitrile solution was radioassayed and analyzed by radio-HPLC.

Fat samples were extracted three times with hexane. The combined hexane solution was partitioned against acetonitrile. The residual solids from the hexane extraction were extracted three times with acetonitrile. The acetonitrile partition from the hexane extracts and the acetonitrile extracts were combined, radioassayed and analyzed by radio-HPLC.

Homogenized GIT sample was extracted three times with acetonitrile. The combined extract was radioassayed and analyzed by radio-HPLC.

Radioassaying

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer. The formed  $^{14}\text{CO}_2$  was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC. The minimum sensitivity of LSC was 0.00055 mg equ/kg for liquid and 0.0006 mg equ/kg for solid samples.

Radio-chromatography and mass spectrometry of the extracts

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 10  $\mu\text{m}$  particles) that was operated with a gradient mixture of 0.1% aqueous acetic acid and methanol. The system was equipped with an UV detector and a radiomonitor.

A combination of liquid chromatography/electrospray mass spectrometry (LC/ESI-MS) was employed for structure evaluation. Mass spectrometry was performed in both the positive and negative ionization modes.

### <sup>19</sup>F-NMR spectroscopy

<sup>19</sup>F-NMR spectra of isolated urine metabolites and reference standards were recorded in methanol solutions. The magnetic field strength was 14.0 Tesla. The observation frequency was 564.717 MHz for <sup>19</sup>F. Chemical shifts were reported as parts per million (ppm) downfield from external trifluoro acetic acid.

## **Findings**

### Recovery of radioactivity in milk, excreta and analyzed organs and tissues (Table 7.2.2.2.2-1)

At study termination, 7 days after the oral dose of <sup>14</sup>C-THNG, the total recovery of the <sup>14</sup>C-label amounted to 91% of the administered dose. The predominant portion of the dose was excreted with the urine (72% of the dose), while only a tenth of the urinary radioactivity was found in the faeces (7% of the dose). Less than 1% of the dose was detected in the (total) milk and 1% of the dose in liver. Kidney, muscle and fat contained less than 1% of the dose.

### Residue levels in milk, blood and organs and tissues (Table 7.2.2.2.2-1)

The highest residue level was detected in the blood amounting to 0.215 mg THNG equ/kg followed by the excretory and metabolizing organs kidney (0.175 mg equ/kg) and liver (0.125 mg equ/kg). Muscle and fat amounted to 0.025 and 0.059 mg equ/kg. The highest residue level in milk was found at the first day after dosing amounting to 0.040 mg equ/kg.

### Composition of residues in milk and dissected organs and tissues (Table 7.2.2.2.2-2)

Milk samples of day one after dosing (morning and evening milk) showed 4 radioactive peaks in the radio-HPLC analysis. None of these peaks could be identified, but thiadone (TH) could definitely be excluded by comparison the HPLC elution times.

In the extracts of liver, kidney, muscle, fat and blood only one radio-peak was detected that could be attributed to thiadone by comparison of the HPLC elution time and by LC/MS.

### Radioactive residues in urine and faeces (Table 7.2.2.2.2-2)

The major portion of renally excreted residues originating from THNG (thiadone-*N*-glucoside) was the oxidation product THNGA (thiadone-*N*-glucuronic acid, 37% of the dose) and the original test compound THNG conjugated with an additional glucuronic acid (THNG-GA, 4% of the dose). Instead of this extra glucuronic acid also sulfuric acid can be linked to form THNGSA (7% of the dose). In these conjugates, the C-N bond between thiadone and endocons remained intact as indicated by <sup>19</sup>F-NMR analysis. A small portion of free thiadone (TH) was detected in the urine amounting to of 7% of the dose.

In faeces, only 1% of the dose was detected as the oxidation product THNGA and another 1% of the dose as free thiadone (TH).

## **Conclusion**

Following administration of the radiolabelled thiadone-*N*-glucoside (THNG) to a lactating goat the radioactive residue was well absorbed and almost completely excreted. The main route was through the urine, with a renal-to-fecal excretion ratio of 10:1. The main metabolic conversion of THNG (in urine) was the oxidation of the glucoside endocon to glucuronic acid and an additional conjugation with glucuronic or sulfuric acid. None of the conjugated metabolites were formed from free thiadone (TH).

While free thiadone was detected in edible tissues of the goat, the residue levels were low at 1x feeding level as the goat in this study was administered with an exaggerated dose. No free thiadone was detected in the milk.

The proposed metabolic pathway of THNG in the goat was derived from the metabolites in urine. The major detoxification proceeded initially through oxidation and conjugation reactions of THNG prior to excretion. The pathway is shown in Figure 7.2.2.2-1.

**Table 7.2.2.2.2-1: Distribution of radioactive residues in excreta, milk and organs and tissue of a goat 7 days after a single oral dose of [thiadiazole-2-<sup>14</sup>C]thiadone-*N*-glucoside (THNG) at a feeding level of 16.3 mg/kg feed/day (given in % of dose and mg equ. of THNG/kg)**

Excreta/Milk/Organ/Tissue	Residue level	
	% of dose	[mg equ/kg]
Urine (total)	72	-
Faeces (total)	7	-
Milk (total)	< 1	max. 0.040 at the first day
Liver	1	0.125
Kidney	< 1	0.175
Muscle	< 1	0.025
Fat	< 1	0.059
Gastrointestinal tract	3	0.057
Bile	< 1	0.014
Blood	2	0.215
Residual carcass*)	6	0.001
Cage wash	< 1	-
Total recovery	91	-

\*) Radioactivity in the residual carcass was estimated based on the carcass weight and the average residues in muscle and fat

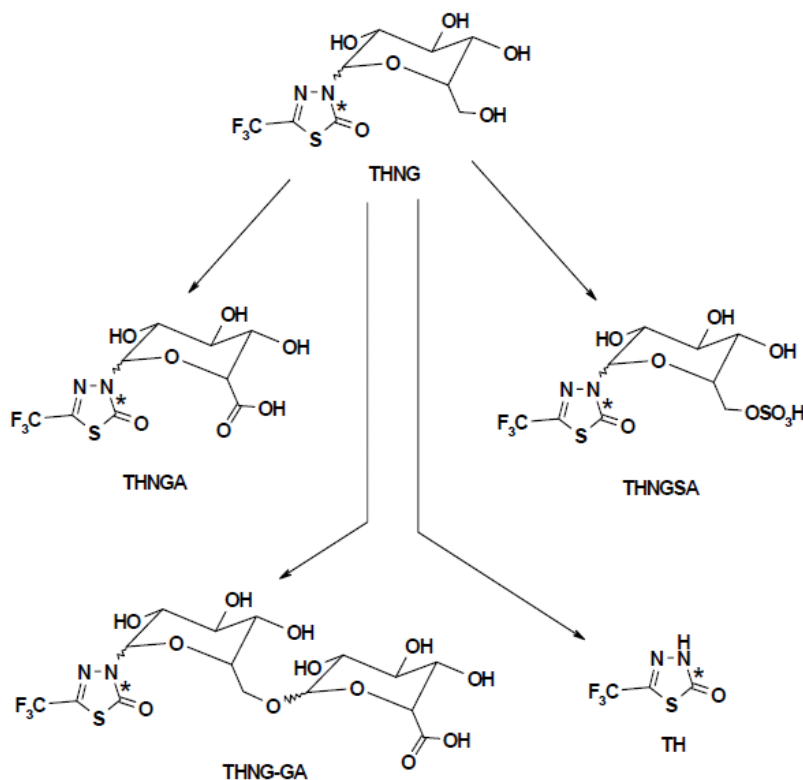
**Table 7.2.2.2.2-2: Radioactive residues in excreta, milk and organs and tissue of a goat 7 days after a single oral dose of [thiadiazole-2-<sup>14</sup>C]thiadone-*N*-glucoside (THNG) at a level 16.3 mg/kg feed/day (given in % of dose or % of TRR in milk/organ/tissues)**

	Residue component				
	THNGA + THNG-GA <sup>#</sup> (M24)	THNG-SO <sub>3</sub> H	THNG (M25)	TH (M9)	unknowns
<b>Excreta/carcass</b>	[% of dose]				
Urine (total)	41	7	12	7	6
Faeces (total)	1	-	-	1	-
Residual carcass	-	-	-	6	-
<b>Milk/organ/tissue</b>	[ % of TRR in milk/organ/tissue]				
Milk (total)	-	-	-	-	100 (4 peaks)*)
Liver	-	-	-	92	-
Kidney	-	-	-	90	-
Muscle	-	-	-	95	-
Fat	-	-	-	48	-
Blood	-	-	-	90	-

<sup>#</sup>) mixture of the two components THNGA (37%) and THNG-GA (4%)

\*) The radiopeaks in milk did not co-eluate with TH

**Figure 7.2.2.2.2-1: Proposed metabolic pathway of thiadone-N-glucoside in the lactating goat**  
(concluded from the metabolites observed in urine)



\* position of  $^{14}\text{C}$ -label

### Metabolism of trifluoroacetic acid in lactating goat

Under natural, physiological and environmental conditions TFA is dissociated and appears as TFA salt. The counter cation depends on the chemical surrounding and is, thus, not defined. Therefore, TFA is expressed as the parent compound of the salts, i.e. as TFA-acid, keeping in mind that a TFA salt was administered to the animals.

<b>Report:</b>	KCA 6.2.3/05, [REDACTED] 2013; M-444459-01-1
<b>Title:</b>	[1- $^{14}\text{C}$ ]Trifluoroacetic acid - Metabolism in the Lactating Goat.
<b>Document No:</b>	M-444459-01-1
<b>Report No:</b>	EnSa-12-0628
<b>Guidelines and data requirements</b>	OECD guideline 503, Metabolism in Livestock, adopted 8-January-2007, US OPPTS guideline 860.1300, Nature of Residues – Plants, Livestock, 1996 Compliant with EU Regulation (EC) No. 1107/2009
<b>GLP</b>	yes

### Executive Summary

A metabolism study with  $^{14}\text{C}$ -labelled Na-TFA was conducted with a lactating goat as TFA revealed to be a major metabolite in plants that were treated with flufenacet and are intended as ruminant feed. This study is needed for a dietary risk assessment including food of animal origin to address the transfer of TFA residue from feedstuffs to milk and edible animal tissue.

TFA was orally administered per gavage as  $^{14}\text{C}$ -Na-TFA to the goat for five consecutive days with one dose per day. The dose level expressed as trifluoroacetic acid, TFA, was 0.50 mg/kg bw/day corresponding to 11.9 mg TFA/kg dry feed/day.

The radioactive residues in milk reached a steady state at approximately 30 hours after the first dose amounting to a plateau level of 0.102 mg parent equivalents/kg (mg equ/kg). Five days after the first administration the goat was slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 0.091 mg equ/kg in fat, 0.347 mg equ/kg in muscle, 0.551 mg equ/kg in liver and 0.967 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analyzed by radio-HPLC (reversed phase) and radio-TLC (straight phase). All radio-chromatograms showed only one chromatographic  $^{14}\text{C}$ -peak. Co-chromatography with authentic  $^{14}\text{C}$ -TFA using two chromatographic methods with different modes of separation unambiguously identified the radioactive peak as TFA. No other radioactive peak appeared in any sample. Therefore, the conclusion is drawn that the total radioactivity in milk, organs and tissues consisted of the unchanged TFA.

By comparison of the residue levels in feed, milk, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: milk (at plateau): 0.0086; muscle: 0.0292; fat: 0.0076; liver: 0.0463; kidney: 0.0813.

## Material and methods

### Test Material

Structural formula	<p>* denotes the <math>^{14}\text{C}</math> label</p>
Chemical name	Sodium trifluoroacetate
CAS RN	2932-18-4
Empirical formula	$\text{C}_2\text{F}_3\text{NaO}_2$
Company code	BCS-AZ56567
Molar mass (non-labelled)	136.01 g/mol
Label	1- $^{14}\text{C}$
Specific radioactivity	4.08 MBq/mg = 110.14 $\mu\text{Ci/mg}$
Radiochemical purity	>98% by TLC and HPLC (radio-detection)
Remark	<p>Trifluoro acetate appeared as anion under physiological and environmental conditions. The corresponding cation depends on the chemical surrounding and, thus, is not defined. Therefore, the residue levels of trifluoro acetate are expressed as the parent substance trifluoro acetic acid (TFA). A conversion is conducted via the ratio of the molar masses:</p> $\text{MM (trifluoroacetic acid)} / \text{MM (sodium trifluoro acetate)} = 114.02/136.01 = 0.8383$ <p>The specific radioactivity of the respective trifluoroacetic acid (TFA) is therefore: <math>4.08 \text{ MB/mg} / 0.8383 = 4.87 \text{ MBq/mg}</math></p>

### Test Animal

Species	Goat ( <i>Capra hircus</i> )
Breed	“Weiße deutsche Edelziege”
Sex, number	One female lactating goat
Body weight	52 kg at first administration, 51 kg at sacrifice
Age	Approx. 15 months
Acclimatization	Two week before administration
Housing	Stainless steel metabolism cage, 18°C, approx. 60% rel. humidity, 12/12 hours light/dark cycle, 10-15 air changes per hour
Feed and water	Ruminant feed, hay, hay pellets, carrot, <i>ad libitum</i> Tap water from local supplier, <i>ad libitum</i>
Health status	Acceptable according to veterinary investigation

#### Preparation of the dosing mixtures and administration

Aliquots of the solid radiolabelled test substance were filled into five gelatin capsules. The sealed capsules were stored at  $\leq -18^{\circ}\text{C}$  until administration. Remaining test substance was used for identification via LC-MS/MS and to demonstrate the storage stability during the dosing period via radio-TLC. One capsule per day was orally administered in the morning for five succeeding days using a capsule applicator. The average daily dose amounted to 30.9 mg sodium trifluoroacetate (corresponding to 25.9 mg trifluoroacetic acid, TFA). Referred to the daily feed consumption and the body weight, this dose corresponded to a dose level of 11.9 mg TFA/kg dry feed or 0.50 mg TFA/kg bw/day. This dose was tolerated without any observable toxicological effects.

#### Collection of milk, urine and faeces

The goat was milked in the morning immediately prior to each administration, and eight hours after administration and directly before sacrifice. The collection intervals for milk sampling were: 0-8, 8-24, 24-32, 32-48, 48-56, 56-72, 72-80, 80-96, and 96-120 hours after the first administration. The milk samples were weighed, radioassayed *via* liquid scintillation counting (LSC) and stored at  $\leq -18^{\circ}\text{C}$  for 97 days.

Urine and faeces were collected on a daily basis. Urine was collected in plastic vessels under dry ice cooling. The faeces samples were homogenized after addition of water to yield a wet paste. Aliquots of the excreta were radioassayed.

#### Sacrifice and collection of organs and tissues

Six hours after the last dose, the goat was sedated and anaesthetized by injection of Xylazin/Rompun, Ketamin and Pentobarbital-Na. Under deep anaesthesia, the animal was exsanguinated by cannulating the jugular vein and finally terminated by intracardiac injection of the veterinary drug "T 61®". Then, the goat was slaughtered and the following organs and tissues were dissected and stored at  $\leq -18^{\circ}\text{C}$  until analysis (103 - 124 days): round and loin muscle, omental and perirenal fat, liver (without gall bladder), and kidneys.

#### Radioassaying and processing of samples

Radioactivity measurements (radioassaying) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted using a sample oxidizer, the formed  $^{14}\text{CO}_2$  was absorbed in an alkaline scintillation cocktail and the resulting solution radioassayed by LSC.

For metabolism investigations, a composite sample of milk collected from 0 h to 102 h (time of sacrifice) after the first administration and composite samples of muscle (loin and round muscle) and fat (perirenal and omental) were prepared. The composite milk, muscle and fat samples and the complete liver, both kidneys and one faeces sample (72 – 96 h) were thoroughly homogenized and kept frozen until extraction. Each sample (except fat) was extracted with acetonitrile/ water (8/2, v/v) and pure acetonitrile using a high-speed stirrer. The fat was extracted with mixtures of n-heptane and acetonitrile/water (8/2, v/v) also using a high-speed stirrer followed by separation of the heptane and the aqueous layer. All acetonitrile/water extracts were concentrated and analyzed by radio-HPLC and radio-TLC.

#### Radio-chromatography and mass spectrometry of the extracts

Radio-HPLC was conducted using a reversed-phase column (RP18, 250 x 4.6 mm, 5  $\mu\text{m}$  particles) that was operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at  $40^{\circ}\text{C}$ . The system was equipped with an UV detector (254 nm) and a radiomonitor with a solid glass scintillator (cell volume 370  $\mu\text{L}$ ). The limit of quantification (LOQ) was derived from background noise level of the baseline and the highest peak in the chromatogram. It ranged from 0.001 mg equ/kg (milk, fat, kidney) to 0.005 mg equ/kg (liver). Column recovery was determined by comparison of injected and eluted radioactivity. In each case, it accounted for 96.2 – 99.6%.  $^{14}\text{C}$ -labelled TFA was co-injected to identify the residues in the samples.

Radio-TLC was conducted on a silica gel TLC plate (20 x 20 cm) that was developed with a solvent mixture of ethyl acetate/2-propanol/water/acetic acid (65/24/22/1, v/v/v/v). Following development the radioactive spots were detected by radioluminography via exposure of an imaging plate to the radioactive spots.

The detection limit was approximately 5-10 dpm/spot after an exposure period of at least 14 hours.  $^{14}\text{C}$ -TFA was also used as reference standard.

The test substance TFA was identified by LC-MS/MS consisting of anion exchange chromatography and a high resolution mass spectrometer. For ion exchange chromatography a Dionex column was eluted with an aqueous solution of 20 mmol KOH as liquid phase. The mass spectrometer was operated in the mode of electro-spray ionization. This test substance was also used as reference standard in radio-HPLC and radio-TLC of the extracts.

## Findings

### Recovery of radioactivity in milk, excreta and analyzed organs and tissues

Six hours after the last of five oral doses of 0.50 mg/kg bw/day of  $^{14}\text{C}$ -labelled TFA approximately 69% of the total radioactivity was recovered in milk, excreta, muscle, fat, liver and kidney. The remaining 31% of the total dose were assumed to be associated with the gastro-intestinal tract and the remaining body. 47.3% of the total dose was excreted with the urine and 15.1% with the faeces. 1.14% of the total dose was secreted into the milk and 5.1% were detected in the dissected edible organs and tissues with 4.1% of the dose being associated with the muscular tissue (assuming 30% of the body weight to the muscular mass).

### Radioactive residues in the milk

The total radioactive residues (TRR) in the milk ranged from 0.079 mg equ/kg to 0.145 mg equ/kg in the collection period 48 to 80 hours after the first administration. At the time of sacrifice, a further increase to 0.171 mg equ/kg was observed due to the shorter time interval between the last dosing and sampling (ca. 6 hours). The time course of radioactivity in milk showed a typical diurnal pattern with temporal peaks eight hours after each administration and sinks shortly before the next dosing (Table 7.2.2.2.2-3). A plateau level was reached approximately 30 hours after the first administration. This level was calculated as mean value of the mass weighted daily averages of the milk samples between the second and the fourth administration. The resulting steady state level in milk amounted to 0.10 mg equ/kg. (The residue levels of the first day were excluded from the plateau calculation since the residues were still increasing at the beginning of milk collection. The residue level of the last milk sample was also excluded since the interval between dosing and milking was shorter than at the other days and a second milk sample was not available due to slaughtering).

### Radioactive residues in dissected organs and tissues

The TRR in edible organs and tissues ranged from fat amounting to 0.091 mg equ/kg (mean of perirenal and omental fat) to kidney amounting to 0.967 mg equ/kg (Table 7.2.2.2.2-4). The radioactivity concentrations of the total muscle and fat referred to 4.08% and 0.43% of the total dose assuming a value of 30% and 12% of the body weight for these tissues, respectively. Altogether, the test radioactive residues in all dissected organs and tissue samples accounted for about 5.14% of the total dose.

### Identification of the radioactive residues

Radio-HPLC and radio-TLC profiles of the extracts of all samples (milk, liver, kidney, muscle, fat, urine and faeces) showed only one polar radioactive peak. Co-chromatography with the reference standard  $^{14}\text{C}$ -TFA resulted in the same single peak. No other peak could be observed. This peak was unambiguously identified as radiolabelled TFA since two chromatographic systems with different separation modes (reversed phase HPLC and straight phase TLC) were used for co-chromatography. Therefore, the total radioactivity in all samples represented unchanged TFA. The rate of identification in the samples was excellent amounting to 98.6 – 100% of TRR in all extracts.

### Transfer factors of residue transfer of TFA from animal fodder to food of animal origin

The TFA transfer factors (TF) were calculated as ratio between the radioactive residues in animal fodder and the total radioactive residues in milk, and edible organs and tissues of the goat. Any correction for formation of transformation products of TFA is not needed as total radioactive residue was represented by the administered test substance (see before). These transfer factors ranging from 0.01 (milk, fat) to 0.08 (kidney) are listed in detail in Table 7.2.2.2.2-5.

## Conclusion

Following repeated oral administration of  $^{14}\text{C}$ -labelled sodium trifluoroacetate (TFA-Na) to a lactating goat for five consecutive days at a dose level of 0.50 mg TFA-acid/kg bw/day (corresponding to 11.9 mg TFA-acid/ kg dry feed/day) the radioactive residues in milk reached a steady state at approximately 30 hours after the first dose amounting to a plateau level of 0.102 mg equ/kg. Five days after the first administration the goat was slaughtered and radioactive residues were determined in edible organs and tissues. These residues accounted for 0.091 mg equ/kg in fat, 0.347 mg equ/kg in muscle, 0.551 mg equ/kg in liver and 0.967 mg equ/kg in kidney. The samples were extracted with acetonitrile/water and the extracts analyzed by radio-HPLC (reversed phase) and radio-TLC (straight phase). Identification of the radioactivity in all samples using co-chromatography with authentic  $^{14}\text{C}$ -TFA and two chromatographic methods with different modes of separation generally showed only one chromatographic peak that was unambiguously identified as TFA. No other radioactive peak appeared in any sample. As a conclusion it can be stated that TFA is metabolically stable. It was rapidly excreted as not more than 5% of the total dose was detected in the dissected organs and tissues 6 hours after administration of the last dose.

By comparison of the residue levels in feed, milk, organs and tissues the following transfer factors for the residue transfer of TFA from animal fodder to food of animal origin could be derived: milk (at plateau): 0.0086; muscle: 0.0292; fat: 0.0076; liver: 0.0463; kidney: 0.0813.

**Table 7.2.2.2-3: Radioactive residues in milk of a goat administered with  $^{14}\text{C}$ -TFA at a dose level of 11.9 mg TFA/kg dry feed/day**

Time schedule after the first administration [hours]	Number of administration	Weight of milk sample [kg]	Residue level in milk sample [mg equ/kg]	Residue level in milk, daily average [mg equ/kg]
0	1		-----	
8		1.27836	0.101**)	
24 *)		2.20244	0.057**)	0.073**)
24	2	-----	-----	
32		1.19898	0.132	
48 *)		2.30380	0.079	0.097
48	3	-----	-----	
56		1.10321	0.138	
72 *)		2.36203	0.079	0.098
72	4	-----	-----	
80		1.16649	0.145	
96 *)		2.34357	0.095	0.112
96	5	-----	-----	
102		0.85857	0.171***)	-
<b>Residue plateau in milk (30–96 hours after first administration)</b>				<b>0.102</b>

\*) Milking immediately before the next administration

\*\*) Not used for calculation of the residue plateau in milk since residues are still increasing at the beginning of the collection period

\*\*\*)) Not used for calculation of the residue plateau in milk since period between dosing and milking was shorter (only 6 hours) and no data of the second milking was available.

**Table 7.2.2.2-4: Radioactive residues in organs and tissues of a goat 6 hours after the last of 5 doses of <sup>14</sup>C-TFA at a dose level of 11.9 mg TFA/kg dry feed/day**

Organ/Tissue	Residue level [mg equ/kg]
Liver	0.551
Kidney	0.967
Round muscle (sample)	0.346
Loin muscle (sample)	0.352
Total body muscle *)	0.347
Perirenal fat (sample)	0.064
Omental fat (sample)	0.107
Total body fat *)	0.091

\*) Weighed mean residue levels in total body muscle and fat were calculated from the sample masses of the two types of muscle and fat and the total radioactive residues in that samples, respectively.

**Table 7.2.2.2-5: Transfer factors for residue transfer of <sup>14</sup>C-TFA from animal feed to milk, muscle, fat, liver and kidney of a goat following repeated administration at a dose level of 11.9 mg TFA/kg dry feed/day**

Milk/Organ/Tissue	Residue level [mg equ/kg]	Transfer factor (TF)
Milk (at residue plateau)	0.102	0.0086
Muscle	0.347	0.0292
Fat	0.091	0.0076
Liver	0.551	0.0463
Kidney	0.967	0.0813

### B 7.2.2.3 Rat metabolism study with [thiadiazole-5-<sup>14</sup>C]flufenacet

Please refer to Section CA 5.1.1 of the Flufenacet dossier, Report KCA 5.1.1/01.

██████████ 2012: [Thiadiazole-5-<sup>14</sup>C]Flufenacet: Supportive Experiment for Identification of Metabolites in the Urine of the Rat; unpublished report of Bayer CropScience Comp. No. M-441499-01-1.

The result of this study is summarized in the following.

Following oral administration of [thiadiazole-5-<sup>14</sup>C]flufenacet to rats (1 mg/kg bw) most of the radioactivity was already excreted within 24 hours with renal excretion being the predominant route of elimination. The excretion pattern was similar to that of a former study on the metabolism of radiolabelled flufenacet in the rat<sup>15</sup>. A polar metabolite detected in urine and blood plasma revealed to be trifluoroacetate (M45) reaching a level of approximately 10% of the administered dose. Therefore, it is concluded that this metabolite is covered in toxicological studies of the parent substance.

<sup>15</sup> ██████████ (1995): The metabolism of FOE 5043 in rats. Unpublished report 106665 of Miles Inc., Stilwell, KS, USA, now Bayer CropScience, Comp. No. M-002247-01-1.

#### B 7.2.2.4 Summary of transfer factors for a potential residue transfer of TFA from fodder plants to food of animal origin resulting from livestock animals

For a dietary exposure assessment the potential residues of TFA in food of animal origin have to be included. The transfer of TFA into eggs, milk, meat, liver and kidneys were determined in the metabolism studies on  $^{14}\text{C}$ -TFA in goat<sup>16</sup> and hen<sup>17</sup> described above. TFA transfer factors derived in these studies are presented in Table 7.2.2.4-1.

**Table 7.2.2.4-1: Summary of TFA transfer factors from animal feed to edible commodities of livestock animals**

Edible Commodity	Goat	Hen
	5 x 0.50 mg TFA/kg bw/day 11.9 mg TFA/kg dry feed	14 x 0.50 mg TFA/kg bw/day 7.84 mg TFA/kg dry feed
Milk (plateau)	0.0086	-
Egg (plateau)	-	0.0499
Muscle	0.0292	0.0784
Fat	0.0076	0.0115
Liver	0.0463	0.0969
Kidney	0.0813	0.1713

#### B 7.2.3 Pigs

The same metabolic reactions (or metabolic stability) were observed in rat, goat and hen when feeding the parent substance flufenacet or the main residue components of flufenacet in animal feed, i.e. FOE oxalate or trifluoroacetate. Therefore, an extra metabolism study in pigs is unlikely to provide new information on the nature of residues in food of animal origin and is consequently not required.

#### B 7.2.4 Fish

The main objective of this kind of study was the determination of a potential bioaccumulation of a test substance in fish during long-term exposure in the fishwater. However, the nature of residues of radiolabelled flufenacet in fillet and viscera of the fish was also disclosed in this study following a 28-day uptake of continuously added [fluorophenyl-UL- $^{14}\text{C}$ ]flufenacet with the inflowing water in flow-through study. As this study yields the same information as a metabolism study in fish it can be used as surrogate study according to Section 6.2.5 of the official data requirements (EU) No. 283/2013 of 1-March-2013 in accordance with Regulation (EC) No. 1107/2009. This study has already been submitted in the Ecotoxicology Section of the original dossier under Section Number 8.2.3 for authorization according to EU Directive 91/414/EEC and has been evaluated in the Monograph including addenda.

The study is divided in two sections and reported in two reports. The first report of Gagliano describes the in-life phase and the determination of the steady-state BCF on basis of radioactivity measurements. The second report of Leimkuehler and Moore describes the nature of residues in the fish following uptake of radiolabelled flufenacet from the fish water.

<sup>16</sup> [REDACTED] (2013): [1- $^{14}\text{C}$ ]Trifluoroacetic acid – Metabolism in the Lactating Goat, unpublished report EnSa-12-0628 of Bayer CropScience AG, Comp. No. M-444459-01-1

<sup>17</sup> [REDACTED] (2013): [1- $^{14}\text{C}$ ]Trifluoroacetic acid – Metabolism in the Laying Hen, unpublished report EnSa-12-0648 of Bayer CropScience AG, Comp. No. M-463376-01-1

<b>Report</b>	<b>KCA 6.2.5/01,</b> [REDACTED] 1994; M-003803-01_
Title:	Uptake, Depuration and Bioaccumulation of Phenyl-[ <sup>14</sup> C]FOE 5043 Technical by Bluegill Sunfish ( <i>Lepomis macrochirus</i> ) Under Flow-Through Conditions
Document No:	M-003803-01-1
Report No:	106760 of Miles Inc. Stilwell, Kansas, USA, now Bayer CropScience AG, dated 1994-07-08
Guidelines:	US EPA Guidelines for Pesticide Registration: Subdivision N, Section 165-4 Accumulation in Fish
GLP	yes

<b>Report:</b>	<b>KCA 6.2.5/02,</b> [REDACTED] 1994; M-003804-01_
Title:	Identification of Radioactive Residues of Phenyl-[ <sup>14</sup> C]FOE 5043 in Bluegill Sunfish ( <i>Lepomis macrochirus</i> )
Document No:	M-003804-01-1
Report No:	106577 of Miles Inc. Stilwell, Kansas, USA, now Bayer CropScience AG, dated 1994-07-13
Guidelines:	US EPA Guidelines for Pesticide Registration: Subdivision N, Section 165-4 Accumulation in Fish
GLP	yes

### Executive Summary

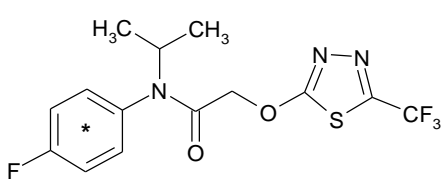
[Fluorophenyl-UL-<sup>14</sup>C]flufenacet was introduced into several aquaria with the inflowing water holding bluegill sunfish in a flow-through experiment for a total exposure period of 28 days. The concentration of the test substance in the aquarium for investigation of fish metabolism was kept constant at a level of approx. 100 µg/L. Several fish were collected after 21- and 28-day of exposure. The total radioactive residues (TRR) in fillet and viscera were essentially the same for both exposure periods amounting to approx. 1.7 (fillet) and 11 (viscera) mg equ/kg. The pattern of metabolites was also nearly identical at both periods. This indicates that residues and the metabolism had reached a steady state.

A total of nine metabolites were identified, but four of these were greater than 5% of TRR in the respective tissue. The data indicate that the primary metabolic pathway starts with a glutathione conjugation of the isopropyl acetanilide moiety (M22) of the parent molecule followed by subsequent formation of FOE cysteine (M23) and its acetylated derivative, the mercapturic acid or FOE acetyl cysteine (M10). A minor metabolic pathway in fish is the hydroxylation of the isopropyl group followed by conjugation with glucuronic acid. A proposal of the metabolic pathway of flufenacet in fish is presented in Figure 7.2.4-1. The same metabolic reactions were, in principle, also found in the laboratory animal rat and in the livestock animals goat and hen.

In separated trials, some fish from other aquaria were collected after different exposure periods and radioassayed for determination of the bioconcentration factor (BCF) This BCF value (applying for a steady state between uptake and elimination) was reached after approx. 7 days of exposure and amounted to 68 – 71 for the whole body.

## Material and methods

### Test Material

Structural formula	 <p style="text-align: right;">* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluorophenyl)-<i>N</i>-isopropyl-2-(5-(trifluoromethyl)-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RNo.	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Water solubility	51 mg/L at pH 6.9 and 20°C <sup>18</sup>
Label	[fluorophenyl-UL- <sup>14</sup> C]Flufenacet
Specific radioactivity	Actually used: 12755 dpm/μg (0.765 MBq/μg, 0.02 mCi/μg) following blending of radiolabelled (66.5 mCi/mmol; 0.183 mCi/mg, 6.77 MBq/mg) and non-labelled Flufenacet
Radiochemical purity	Original: 98.9%, re-analysis by radio-TLC: 95.3%
Chemical purity of the non-labelled test substance	96.8%
Solvent for stock solution	Acetone

### Test Animals

Species	Bluegill sunfish ( <i>Lepomis macrochirus</i> )
Breed	Osaga Catfisheries, Osaga Beach, Missouri, USA
Number	Approx. 95 smaller fish per aquarium at the beginning for BCF determination, also used for investigation of the metabolism in fish; 6 larger fish per aquarium after removal the small fish to support in the disclosure of the metabolites
Body length	Smaller fish: approx. 19 mm; larger fish: 4 – 6 inch (10 – 15 cm)
Acclimatization	Smaller fish: 1 month Larger fish: 4 days
Husbandry	Two 100 L glass aquaria with a standpipe for drainage, filled with 78 L water, temperature 22 ± 2°C, pH 7.1 – 7.5, 16-hour daylight period
Feed	Newly hatched brine shrimp and commercial fish food, daily feeding
Water turn overs in the flow-through system	Approx. 10.5 – 11.2 volumes per 24 hours, the inflowing water passed an ultraviolet sterilizer
Duration (only uptake)	Smaller fish: 28 days at maximum, Larger fish: additional 7 days (1 <sup>st</sup> aquarium), 14 days (2 <sup>nd</sup> aquarium)

### Exposure of fish to radiolabelled test substance

Three 100 L glass aquaria holding initially 150 smaller fish each (body length approx. 19 mm, body weight approx. 0.17 g) were kept in flow-through condition for a total uptake period of 28 days and a subsequent

<sup>18</sup> Ziemer, F., Peschke, C., 2012: Flufenacet (FOE 5043, AE F133402), pure substance: Solubility in distilled water (flask method), unpublished report PA12/059 of Bayer CropScience AG, Comp. No. M-438187-01-1.

depuration period of 14 days (two aquaria with the test substance, one control aquarium without test substance). During the uptake period radiolabelled flufenacet was added to the inflowing water to reach a concentration in the fish water of approx. 100 µg/L. During the depuration period pure water with no test substance was introduced.

Following complete removal of the smaller fish six larger fish (body length approx. 10 – 15 cm) were inserted in each of the two aquaria and exposed to radiolabelled flufenacet in the same way as done with the smaller fish.

#### Collection of fish and extraction of fish

The smaller fish of the BCF trial were sampled after different exposure periods, i.e. 0, 1, 3, 7, 14, 21, and 28 days. They were directly radioassayed (following cutting in suitable pieces) or first dissected into fillet (edible) and viscera (non-edible tissue). Respective fractions were ground to powder under liquid nitrogen using mortar and pestle. The liquid nitrogen was allowed to sublime in a freezer at -20°C. The fillet and viscera samples were also radioassayed to determine the total radioactive residues (TRR) in the whole body, fillet and viscera.

Fillet and viscera samples of collection days 21 and 28 were extracted with methanol and a mixture of methanol and 0.1N hydrochloric acid at room temperature. The methanol extract was partitioned against hexane. The hexane solution was discarded. The methanol fraction was concentrated, centrifuged and analyzed by radio-HPLC.

The larger fish were collected after a 7 (first aquarium) and 14-day exposure (second aquarium). These fish were dissected and their bladders were carefully removed, punctured and drained. The removed urine was centrifuged and analyzed by radio-HPLC.

#### Extraction of fish water

Water samples were taken at the same time as fish were collected. Radioactive residues in these water samples were extracted with dichloromethane, the extracts concentrated to dryness and re-constituted in methanol. Alternatively, radioactive residues in water samples were also extracted by solid-phase extraction using a C18 cartridge. Adsorbed residues were eluted by flushing with methanol. The methanol extracts were concentrated and analyzed by radio-HPLC.

#### Radioassaying of samples

Radioassaying (radioactivity measurements) were conducted by liquid scintillation counting (LSC); aliquots of liquid samples were directly measured, aliquots of solid samples were first combusted and the formed <sup>14</sup>CO<sub>2</sub> was absorbed in an alkaline scintillation cocktail. The minimum counting efficiency (LOD) was derived from the lowest net count rate of the LSC-counter, the specific activity of the test substance and the sample size used for LSC counting. For fish water a LOD of 5.77 x 10<sup>-3</sup> µg equ/L, for fish tissue a LOD of 0.096 µg equ/kg was reported.

#### Radio-HPLC and LC-MS of sample extracts

Radio-HPLC was conducted using RP8 columns (250 x 9.4 cm, particle size 10 µm and 250 x 4.6 cm, particle size 5 µm) operated with gradient mixtures of aqueous 0.1% acetic acid or trifluoroacetic acid and methanol or acetonitrile. The systems were equipped with a radiomonitor with a 400 or 500 µL cell with a solid scintillator.

LC-MS was conducted by a combination of a HPLC system, a radiomonitor and a mass spectrometer. The HPLC system used a RP18 separation column (150 x 4.9 mm, particle size 5 µm) and was operated with gradient mixture of aqueous 0.1% acetic acid and methanol. Determination of the separated compounds was performed by a double focusing mass spectrometer with a thermospray interface.

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## Findings

### TRR in fish and derived BCF values (Gagliano, 1994)

Bluegill sunfish were exposed to dissolved radiolabelled flufenacet in fishwater at a concentration of approx. 100 µg/L for different exposure periods. The total radioactive residue (TRR) in fish tissue amounted to 833 - 2213 µg equ/kg in edible fillet, to 5899 - 10846 µg equ/kg in non-edible viscera and to 3315 - 9900 µg equ/kg in whole fish. Comparing the residue levels in fish tissue and fish water resulted in daily bioconcentration factors (BCF values) of 8.4 – 22.1 for fillet, 59.2 – 111 for viscera and 33.3 – 98.0 for the whole body. The plateau levels (steady-state levels) were already reached after approximately 7 days of exposure.

The mean steady-state BCF for the whole body was determined to 68 (mean BCF of the last four sampling dates 7, 14, 21 and 28 days of uptake) or 71.4 when calculated using the BIOFAC model operating on the basis of an uptake and depuration rate constant.

### TRR in fish water and hydrolytic stability of the test substance (Leimkuehler, Moore, 1994)

Radioassaying of fish water at the different collection days resulted in a radioactivity concentration in the range of 95.9 – 100.0 µg equ/L. Determination of the intact test substance amounting to 86.7 – 95.0 µg/L indicated no significant degradation of the test substance in the aquaria.

### Composition of radioactive residues in fish tissue

The composition of the radioactive residues in viscera and fillet of bluegill sunfish following 21 and 28 day exposure of radiolabelled flufenacet are presented in Table 7.2.4-1 and Table 7.2.4-2. The structures of the metabolites were derived from their mass spectra and by comparison of the retention behavior in reversed phase HPLC. The composition of residues in viscera and fillet was almost identical during the 21 and 28 day exposure indicating a steady state metabolism.

The major metabolites in non-edible viscera were identified as FOE cysteine conjugate (FACS, M23) amounting to approximately 50% of TRR and its acetylated derivative FOE acetyl cysteine (FANACS, “mercapturic acid”, M10) amounting to approximately 24% of TRR. Other four minor metabolites (<10% of TRR) were also identified. The parent substance flufenacet was observed at a low level of approximately 5% of TRR.

The major metabolites in edible fillet proved to be also FOE cysteine conjugate (FACS, M23) amounting to approximately 37% of TRR and FOE acetyl cysteine (FANACS, M10) amounting to approximately 16% of TRR. Eight unknown minor metabolites could be characterized according to their polarity (retention behavior in reversed phase liquid chromatography). The parent compound flufenacet contributed significantly to pattern of residues accounting for 18% of TRR.

## Conclusion

The bioconcentration factor (BCF) of [fluorophenyl-UL-<sup>14</sup>C]flufenacet in bluegill sunfish amounted to 68 – 71 for the whole body based on radioactivity measurements. The respective steady state of uptake and elimination was reached after approx. 7 days of exposure at a concentration of 100 µg/L.

The metabolism of [fluorophenyl-UL-<sup>14</sup>C]flufenacet was investigated in bluegill sunfish after 21 and 28-day exposure in the fish water a concentration of approx. 100 µg/L. The TRR levels in the fillet and viscera were essentially the same for both exposure periods amounting to approx. 1.7 (fillet) and 11 (viscera) mg equ/kg. The pattern of metabolites was also nearly identical at both periods. This indicates that residues and the metabolism had reached a steady state.

A total of nine metabolites were identified, four of these were greater than 5% of TRR in the respective tissue. The data indicate that the primary metabolic pathway starts with a glutathionate conjugation of the isopropyl acetanilide moiety (M22) of the parent molecule followed by subsequent formation of FOE cysteine (M23) and its acetylated derivative, the mercapturic acid or FOE acetyl cysteine (M10). A minor

metabolic pathway in fish is the hydroxylation of the isopropyl group followed by conjugation with glucuronic acid. A proposal of the metabolic pathway of flufenacet in fish is presented in Figure 7.2.4-1.

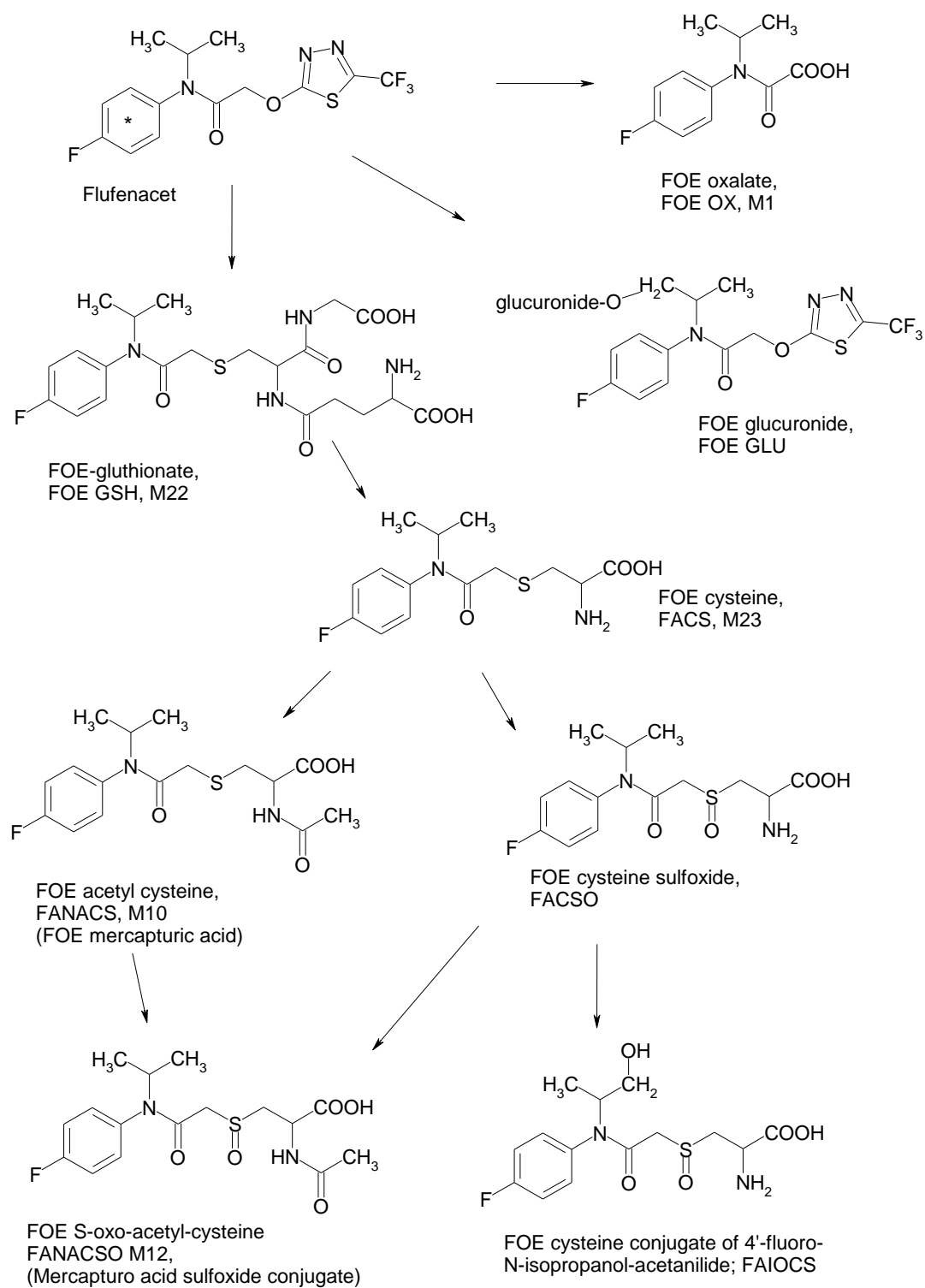
The same principle metabolic reactions were also found in the laboratory animal rat and in the livestock animals goat and hen.

**Table 7.2.4-1: Radioactive residues in viscera of bluegill sunfish following 21 and 28 day exposure of [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a concentration of 100 µg/L fish water in a flow-through study**

Exposure period	21 Days		28 Days	
TRR [mg equ/kg] (after combustion)	10.99		10.22	
<b>Metabolite</b> detected by radio-HPLC	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
FOE isopropyl hydroxy cysteine (FAIOCS) and FOE oxalate (FOE OX, M1)	2.6	0.258	1.8	0.191
FOE cysteine sulfoxide conjugate (FACSO, M39)	3.3	0.327	1.1	0.114
FOE S-oxo-acetylcysteine (FANACSO, M12)	4.4	0.444	0.9	0.097
FOE glutathionate (FOE GSH, M22)	3.5	0.335	2.3	0.239
FOE cysteine conjugate (FACS, M23)	46.9	4.694	54.8	5.719
FOE acetyl cysteine (FANACS, M10)	24.0	2.408	23.3	2.431
FOE isopropanol glucuronide (FOE GLU)	5.2	0.520	4.5	0.467
Flufenacet (FOE 5043, parent substance)	3.8	0.381	5.7	0.599
Unextracted	6.3	0.631	5.6	0.586
Total	100	10.018	100	10.443

**Table 7.2.4-2: Radioactive residues in the fillet of bluegill sunfish following 21 and 28-day exposure of [fluorophenyl-UL-<sup>14</sup>C]flufenacet at a concentration of 100 µg/L fish water in a flow-through study**

Exposure period	21 Days		28 Days	
TRR [mg equ/kg] (after combustion)	1.79		1.76	
<b>Metabolite</b> detected by radio-HPLC	[% of TRR]	[mg equ/kg]	[% of TRR]	[mg equ/kg]
Unknown 1	1.3	0.026	1.5	0.026
Unknown 2	1.4	0.027	2.1	0.036
Unknown 3	1.2	0.022	2.4	0.041
Unknown 4	2.1	0.039	3.0	0.052
FOE cysteine conjugate (FACS, M23)	36.2	0.692	37.3	0.631
FOE acetyl cysteine (FANACS, M10)	17.1	0.326	15.3	0.260
Unknown 5	2.0	0.038	1.9	0.032
Unknown 6	1.4	0.027	1.6	0.027
Unknown 7	1.6	0.030	0.7	0.001
Unknown 8	1.6	0.030	0.8	0.014
Flufenacet (FOE 5043, parent substance)	18.1	0.345	17.6	0.297
Unextracted	16.0	0.308	15.8	0.268
Total identified	69.3	1.326	68.4	1.159
Total	100	1.910	100	1.696

**Figure 7.2.4-1: Proposed metabolic pathway of [fluorophenyl-UL-<sup>14</sup>C]flufenacet of bluegill sunfish**\* <sup>14</sup>C radiolabel

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**B 7.2.5 Summary of the animal metabolism studies**

In the goat Flufenacet is extensively metabolised. The first metabolic step is conjugation with glutathione. Further biodegradation follows the mercapturic acid (acetyl cysteine) pathway, with additional formation of cysteine (M23)- or mercapturic acid (M10) conjugates.

In poultry metabolism of Flufenacet appeared to involve the mercapturic acid pathway resulting in a wide range of methylsulfinyl and methylsulfonyl containing metabolites produced from further metabolism of the cysteine and mercapturic acid conjugates of Flufenacet.

The metabolism studies performed with flufenacet indicate a wide range of metabolites are formed containing the *N*-fluorophenyl-*N*-isopropyl moiety. Therefore, EFSA concluded that for commodities of animal origin, it is desirable to include all metabolites containing the *N*-fluorophenyl-*N*-isopropyl moiety in the residue definition, both for enforcement and risk assessment.

In the EFSA reasoned opinion a detailed assessment is provided on the review of the existing maximum residue levels according to Art 12 of Regulation (EC) no. 396/2005 (2012). The general metabolic pathways in rodents and livestock were found to be comparable (see Figure 7.2.5-1) .

The metabolism of flufenacet in fish was investigated in the context of a bioconcentration study with bluegill sunfish. [Fluorophenyl-UL-<sup>14</sup>C]flufenacet was introduced into the inflowing water to aquaria with the fish to achieve a concentration of 100 µg/L. The principle metabolic pathway was the glutathione conjugation of the acetanilide moiety of the molecule followed by further metabolic conversions as already observed in rat, goat, hen and plants. The residue levels in edible tissue and viscera were low as documented by the low bioconcentration factor of 68-71 for the whole body.

Since the parent compound degrades rapidly in plants and is not detectable in animal feeding items the metabolism study using [fluorophenyl-UL-<sup>14</sup>C]Flufenacet oxalate (M01) provides the most relevant information. Oral administration of this oxalate to ruminant and poultry showed its metabolic stability. Flufenacet oxalate is essentially not metabolised by the animal. This metabolic stability was confirmed by a bio-availability study of flufenacet oxalate in rats. The low residue levels in tissue, milk and eggs suggest that flufenacet oxalate is minimally absorbed and rapidly excreted. Following oral administration of radiolabeled flufenacet oxalate to three rats at a dose rate of approx. 1 mg/kg bw 19 – 37% of the dose was excreted with urine and 61 – 80% was excreted with faeces as unchanged flufenacet oxalate.

Another major plant metabolite in animal feeding items was detected as thiadone-*N*-glucoside (M25, THNG). This metabolite was observed after treatment of [thiadiazole-2-<sup>14</sup>C]Flufenacet to plants. Oral administration of radiolabelled M25 to a goat at an overdosed feeding rate indicated low residues in milk and edible organs and tissues. Free thiadone formed by de-conjugation was the main residue component in kidney, liver and muscle, but not in milk. However, the residue level of thiadone is negligible (< 0.01 mg/kg) if the overdose is transformed to a 1x feeding level.

New plant metabolism studies with [thiadiazole-5-<sup>14</sup>C]flufenacet in primary and succeeding plants revealed trifluoroacetate (M45) as a major metabolite in edible plant parts and in plant parts intended as feeding stuff for livestock animals. For a complete dietary risk assessment including residues in food of animal origin, a potential residue transfer of trifluoroacetate from feeding items to food of animal origin has been investigated.

Therefore, metabolism studies on <sup>14</sup>C-labelled trifluoroacetate in goat and hen were conducted indicating the metabolic stability and a low residue transfer of trifluoroacetate to milk, eggs and edible organs and tissues. The respective transfer factors were low and did not indicate any accumulation in milk, eggs and edible organs and tissues of livestock animals.

Parent Flufenacet is not detectable in any crop commodity (including livestock feed). Although livestock metabolism studies were conducted using [thiadiazole-2-<sup>14</sup>C]flufenacet and [fluorophenyl-UL-<sup>14</sup>C]flufenacet, the metabolism studies using the main plant metabolites in livestock feed are considered to

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provide the relevant information. Therefore, metabolism studies were conducted with the main metabolites in feed, i.e. FOE-oxalate (M01) and thiadone-N-glucoside (M25) on goat and hen:

M01 was stable and was detected in trace levels in milk and egg and in measurable levels in consumable tissues. All transfer factors (TF) were  $< 1$ . M25 was not observed in milk and tissues. Low levels of the aglycon thiadone (M09) were detected in fat, muscle, kidney and liver. All TF were  $< 1$ .

[illegible]

Red numbers indicate the bioavailable portion in the rat, given in % of the oral dose.

### B.7.3. MAGNITUDE OF RESIDUE TRIALS IN PLANTS

The herbicide flufenacet is mainly used to control annual grasses and broad-leaved weeds in cereals (wheat, rye, triticale, barley and oat). It may be applied either pre- or post-emergence of the cereals. Flufenacet is usually co-formulated with other herbicides such as diflufenican. The representative formulation for the renewal of the approval of flufenacet is 'Flufenacet + Diflufenican SC 600', a soluble concentrate formulation containing 400 g/L of flufenacet and 200 g/L of diflufenican.

The product 'Flufenacet + Diflufenican SC 600' was also the representative formulation for evaluation of diflufenican in the EU peer review process (2008).<sup>19</sup>.

During initial Annex I submission representative uses included cereals, corn, soybeans and sunflower while those for renewal of approval of flufenacet are limited to cereals only.

#### B.7.3.1 Cereals (wheat, rye, triticale, barley and oats)

According to the 'guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs', SANCO 7525/VI/95 rev 9 (March 2011), extrapolation of residue data obtained from any of the crops (wheat, rye, triticale, barley, oats) for an active substance is possible if the use pattern involves treatments early in the growing season (last application before consumable parts of the crop have started to form).

Therefore combined data sets obtained from residue studies on wheat and barley are reported in this chapter in order to support flufenacet uses.

#### Representative uses for renewal of approval of flufenacet

The representative uses supported for the renewal of approval for flufenacet are summarised in Table 7.3.1-1.

**Table 7.3.1-1: Summary of the representative uses supported for renewal of approval for flufenacet in the product 'Flufenacet + Diflufenican SC 600'**

Crop	Region*	F, G or I**	Maximum Number of Applications	Growth stage at application	Maximum Rate flufenacet (g a.s./ha)	Minimum PHI (days)
Cereals (winter wheat, winter barley, winter rye)	EU-N	F	1	Early post-emergence BBCH 10-13 (autumn use)	240	n.a.
Cereals (winter wheat, winter barley, winter rye)	EU-N	F	1	Pre-emergence; early post-emergence BBCH 00-22	120	n.a.
Cereals (wheat, barley)	EU-S	F	1	Early post-emergence BBCH 11-13	240	n.a.
Cereals (wheat, barley)	EU-S	F	1	Early post-emergence BBCH 11-13	160	n.a.

\* EU-N northern Europe EU-S southern Europe \*\* F Field; G Greenhouse; I Indoor

n.a. not applicable, the PHI is covered by the vegetation period of the crop from treatment to harvest.

<sup>19</sup> In the initial version of the Annex II dossier that was issued in November 2003 a different representative use was supported, namely autumn application to winter cereals at the rate of 187.5 g a.s./ha up to the growth stage BBCH 25 (5 tillers detectable). This use corresponded to autumn application of the formulation JAVELIN® (500 g/L isoproturon + 62.5 g/L diflufenican). Autumn application of HEROLD®SC600 (Flufenacet + Diflufenican SC 600) in winter cereals was proposed as a second representative use and dealt with separately in an Annex III dossier. In January 2004 the Rapporteur Member State and Bayer CropScience agreed to consider only the use of HEROLD®SC600 as the representative use for the EU review.

Representative use included in the Annex II dossier and evaluated for Annex I inclusion

**The representative use** considered during the EU review of flufenacet (and taken into account for Annex I inclusion of the active substance) **is pre-emergence/early post-emergence application to winter cereals (wheat, rye, triticale, barley) in autumn at the rate of 240 g as/ha.** Since the use pattern referred to autumn application no specific growth stage for the crop was defined for the latest possible application. The application is typically made pre-emergence or during leaf development or tillering.

The representative product in the Annex II dossier to support the critical GAP for flufenacet in wheat, rye, triticale and barley at the European level was a straight formulation WG 60, containing 60% flufenacet. The use was supported in the north European climatic zone. The use evaluated with the Annex II dossier corresponds to the critical GAP for flufenacet in northern Europe.

The GAP of the representative use in cereals supported with the Annex II dossier and taken into account for Annex I inclusion is summarised in Table 7.3.1-2.

**Table 7.3.1- 2: Summary of the representative use of Flufenacet WG 60 considered for Annex I inclusion of the active substance flufenacet**

Crop	Region *	F, G or I**	Growth stage	Maximum Number of Applications	Maximum Rate (g as/ha)	Minimum PHI (days)
Winter wheat Winter barley Winter rye	EU-N	F	pre-emergence to early post emergence (autumn)  2 <sup>nd</sup> leaf stage of weeds	1	240	n.a.

\*EU-N: northern Europe \*\*F Field; G Greenhouse; I Indoor.

n.a. : not applicable. The pre-harvest interval covers the vegetation period of the crop until harvest.

#### **B.7.3.1.1 Studies evaluated during Annex I submission and reconsidered for renewal of active substance approval**

##### References:

<b>Report:</b>	Jersch-Schmitz. S., Seym. M. (1995): Determination of residues of <i>FOE 5043 60 WG</i> in/on winter wheat and winter barley following early post-emergence spray application in Germany, France and the Netherlands; Bayer AG, unpublished report no.: RA-2054/93 of October 10, 1995
<b>Guideline:</b>	Guidelines for the Establishment of Community Maximum Residue Levels (MRLs) of Plant Protection Products in Food and Feedstuff's of Plant and Animal Origin; prepared by Dr. J.-R. Lundehn for the Commission of the European Communities; version as of January 1993
<b>GLP:</b>	yes (certified laboratory)

<b>Report:</b>	Seym, M. (1996): Determination of residues of <i>FOE 5043 60 WG</i> in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany and France; Bayer AG, unpublished report no.: RA-2008/94 of March 25, 1996
<b>Guideline:</b>	Guidelines for the Establishment of Community Maximum Residue Levels (MRLs) of Plant Protection Products in Food and Feedstuff's of Plant and Animal Origin; prepared by Dr. J.-R. Lundehn for the Commission of the European Communities; version as of January 1993
<b>GLP:</b>	yes (certified laboratory)

A total of 18 residue trials on winter barley (7 trials, 1 trial yielding green plant material only), winter rye (2 trials) and winter wheat (9 trials), which were performed at different sites in northern Europe during the 1993/94 and 1994/95 growing seasons, are reviewed in the Annex II Section 4 for flufenacet. The plants were treated post-emergence between mid-October and mid-March at growth stages ranging from BBCH 11 (first leaf unfolded) to BBCH 25 (5 tillers detectable). A straight WG formulation containing 60% w/w of flufenacet (WG 60) was applied at a nominal rate of 0.4 kg/ha, which corresponds to 240 g as/ha. In two trials the actually achieved rate slightly differed from the nominal rate, at either

188 g as/ha (ca. 22% less) or 260 g as/ha (ca. 8% more). Harvest was between 120 and 271 days after application. Residues were determined at various development stages of the treated plants.

- Depending on the growth stage and season when the treatment was performed, the residues in the green plants at the growth stage BBCH 29 (end of tillering) ranged between < 0.05 and 0.25 mg/kg and were < 0.05 mg/kg at the growth stage BBCH 51 (beginning of heading).
- The residues of flufenacet in grain and straw at harvest were always below the respective limit of quantification, i.e. 0.05 mg/kg in grain and 0.10 mg/kg in straw.

The residue trials considered to grant Annex I inclusion of flufenacet are summarized in Table 7.3.1.1-1 below.

**Table 7.3.1.1-1 Number of residue trials conducted per geographical region and vegetation period considered for Annex I inclusion of the active substance flufenacet**

Crop	Formulation	Year	Application rate (g as/ha)	Growth stage at application	Country (No. of trials)	Report No.	Annex II Baseline dossier reference Report
<b>Northern Europe</b>							
Winter barley	WG 60 (60 %)	1993/94	240 240	BBCH 12-21 BBCH 22-24	Germany (3**) France N (2)*	RA-2054/93	KCA 6.3.1/02 Jersch-Schmitz, S.; Seym, M.; 1995; M-002284-01-2
Winter wheat	WG 60 (60 %)	1993/94	240 240 240	BBCH 11-12 BBCH 13-22 BBCH 12	Germany (3) France N (2)* the Netherlands (2)*	RA-2054/93	KCA 6.3.1/02 Jersch-Schmitz, S.; Seym, M.; 1995; M-002284-01-2
Winter wheat	WG 60 (60 %)	1994/95	240 186	BBCH 21 BBCH 13	Germany (1) France N (1)	RA-2008/94	KCA 6.3.1/01 Seym, M.; 1996; M-002280-01-2
Winter barley	WG 60 (60 %)	1994/95	240 260	BBCH 13 BBCH 21	Germany (1) France N (1)	RA-2008/94	KCA 6.3.1/01 Seym, M.; 1996; M-002280-01-2
Winter rye	WG 60 (60 %)	1994/95	240 240	BBCH 21 BBCH 25	Germany (2)	RA-2008/94	KCA 6.3.1/01 Seym, M.; 1996; M-002280-01-2

\*application carried out in March \*\* only green material, but no grain and straw were sampled in one trial

The samples from the trials supporting the representative use of Flufenacet WG 60 dealt with in the Annex II dossier were analysed for residues of flufenacet according to the method 00346 (Seym, M.; 1995; M-018864-02), which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group with a limit of quantification of 0.05 mg/kg in grain and green plant material and 0.1 mg/kg in straw.

Before analysis the grain and straw samples were stored frozen for up to 250 days (8.5 months), while samples of green material were stored for a maximum storage period of 350 days (12 months). The maximum storage periods are covered by the storage period investigated in the storage stability.

Concurrent recoveries were performed during the analysis of the study samples. Recovery means were within the range of 70-110% in grain, straw and green material. The relative standard deviation was < 20% for all sample materials and at all fortification levels.

Residues in the control samples were below the respective LOQs (0.05 mg/kg for grain and green material and 0.1 mg/kg for straw). The residue levels in the samples of green plant material, grain and straw from the trials supporting the representative use are summarised in Table 7.3.1.1-2.

**Table 7.3.1.1-2: Summary of flufenacet residue data supporting the representative use considered for Annex I inclusion of the active substance flufenacet**

Application	Sample material	n	Residue level (mg/kg)		
			Min.	Max.	Median
Northern Europe					
240 (186-260) g as/ha at latest BBCH 25 (application November to March)	Grain	17	< 0.05	< 0.05	< 0.05
	Straw	17	< 0.1	< 0.1	< 0.1
	Green material (BBCH 51)	18	< 0.05	< 0.05	< 0.05

The residue trials considered to grant Annex I inclusion of flufenacet support application of flufenacet to cereals at the rate of 240 g as/ha at pre- or early post emergence growth stages up to mid of tillering. The applications were performed between November and March (BBCH 11 to 25) and were considered suitable to support the autumn/winter use of the product Flufenacet WG 60.

Details of these studies are presented in greater detail at the end of this document (Appendix 1)

#### **B.7.3.1.2 Summary of the residue trials supporting the representative use for the Annex I renewal process**

The representative uses supported for the renewal of approval for flufenacet are summarised in Table 7.3.1-1 above.

For the northern climatic zone, the critical use pattern of the representative product ‘Flufenacet + Diflufenican SC 600’ involves the same application parameters relative to flufenacet as ‘Flufenacet WG 60’ considered to grant Annex I inclusion. For both products the maximum supported application rate of flufenacet amounts to 240 g as/ha.

The trials reviewed in the Annex II dossier of flufenacet were performed using a WG formulation which is known to produce comparable residue levels to SC formulations. Therefore, both formulation types can be used interchangeably to support either of the products (cf. ‘guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs’, SANCO 7525/VI/95 rev 9 (March 2011) and ‘OECD guideline for the testing of chemicals’-crop field trial, 509).

Thus, the residue trials reviewed in the Annex II dossier of flufenacet are considered to adequately support the representative use of ‘Flufenacet + Diflufenican SC 600’ in northern Europe. In principle, no further trials are required.

Supplementary trials are available to support the representative use patterns relevant for renewal of approval in northern and southern European climatic zones. The studies were conducted using mixture products, either a 2 way mixture with diflufenican or a product containing 3 active substances (i.e. flufenacet, diflufenican and flurtamone). The supplementary studies cover application rates from 110 to 254 g as/ha addressing the representative uses at the corresponding rates. An overview on the studies is compiled in Table 7.3.1.2-1. In principle, the studies involving application rates at 240 g as/ha (actual 220-254 g as/ha) are considered adequate to also support use patterns involving lower rates of flufenacet by establishing the risk envelope for the active substance in both climatic regions.

In order to support the active substance flufenacet only residue data pertaining to flufenacet are summarized below and in greater detail at the end of this document (Appendix 1).

**Table 7.3.1.2-1: Supplementary residue trials conducted per geographical region and formulation**

Crop	Formula-tion	Year	Applica-tion rate Flufenacet (g as/ha)	Growth stage at application	No. of trials	Report No.	KCA reference Report	Documentation reference number
<b>Northern Europe</b>								
Wheat Barley Rye	FFA+DFFWG 60	1993/94	240	BBCH 13-25	4	RA- 2010/94	KCA 6.3.1/03	M-004451-01- 2
Wheat Barley	FFA+DFF SC 600	2000/01	240	BBCH 13	2	RA- 2144/00	KCA 6.3.1/06	M-058156-01- 1
Wheat	FFA+DFF+FLT SC360	2011	110-120	BBCH 25	2	11-2095	KCA 6.3.1/09	M-459755-01- 1
Barley	FFA+DFF+FLT SC360	2011	120	BBCH 25	2	11-2094	KCA 6.3.1/12	M-460003-01- 1
Wheat barley	FFA+DFF+FLT SC360	2011/12	120	BBCH 22-25	4	12-2001	KCA 6.3.1/10	M-459795-01- 1
<b>Southern Europe</b>								
Wheat Barley	FFA+DFF SC 600	2000/01	240-254	BBCH 13	2	RA- 2144/00	KCA 6.3.1/06	M-058156-01- 1
Barley	FFA+DFF SC 600	2008/09	240	BBCH 13	3	09-2048	KCA 6.3.1/07	M-361495-01- 1
Wheat	FFA+DFF SC 600	2008/09	220-240	BBCH 13- 21	4	09-2052	KCA 6.3.1/08	M-363200-02- 1
Wheat	FFA+DFF+FLT SC360	2011	120*	BBCH 29-30	2	11-2095	KCA 6.3.1/09	M-459755-01- 1
Barley	FFA+DFF+FLT SC360	2011	120*	BBCH 25-29	2	11-2094	KCA 6.3.1/12	M-460003-01- 1
Wheat Barley	FFA+DFF+FLT SC360	2011/12	120*	BBCH 22-25	3 (4**)	12-2002	KCA 6.3.1/11	M-459799-01- 1
Wheat Barley	FFA+DFF WG70	1997	126*	BBCH 13	3	RA- 2153/97	KCA 6.3.1/04	M-012486-02- 1
Wheat Barley	FFA+DFF WG70	1998	126*	BBCH 13	2	RA- 2185/98	KCA 6.3.1/05	M-033163-01- 1

FFA+DFF WG 60: wettable granule formulation containing 40% flufenacet + 20% diflufenican

FFA+DFF SC600 suspension concentrate containing 400 g/L flufenacet +200 g/L diflufenican

FFA+DFF+FLT SC 360: suspension concentrate containing 120 g/L flufenacet +120 g/L diflufenican + 120 g/L flurtamone

FFA+DFF WG 70 wettable granule formulation containing 35% flufenacet + 35% diflufenican

\* residue trials at a rate of 120 g as/ha are considered appropriate to also support the GAP involving 160 g as/ha since the rate is within the acceptable 25% range of comparability (Guideline on comparability, extrapolation, group tolerances and data requirements for setting MRLs<sup>3</sup>, SANCO 7525/VI/95 rev 9)

\*\*One trial was underdosed by 7% and thus out of the acceptable range for comparability of 25% relative to the supported use pattern.

**Table 7.3.1.2-2: Overall summary of supplementary residue data on cereals supporting the representative GAPs for renewal of approval**

Application Rate flufenacet (g as/ha)	Region	Formulation	Crop	Sample material	n	Residue level (mg/kg) flufenacet		
						Min.	Max.	STMR
240	EU-N	FFA+DFF WG 60 FFA+DFF SC 600	wheat, barley	grain	6	<0.05	<0.05	<0.05
				straw	6	<0.10	<0.10	<0.10
110-120	EU-N	FFA+FLT+DFF SC 360	Wheat, barley	grain	8	<0.01	0.022	<0.01
				straw	8	<0.05	<0.05	<0.05
220-254	EU-S	FFA+DFF SC 600	Wheat, barley	grain	9	<0.01	0.05	<0.01
				straw	9	<0.05	0.11	0.06
120-126	EU-S	FFA+FLT+DFF SC 360 FFA+DFF WG 70	Wheat, barley	grain	12	<0.01 <0.05	0.035/ <0.05	0.022
				straw	12	<0.05	0.069	<0.05

EU-N northern Europe

EU-S southern Europe

n: number of trials

FFA+DFF WG 60 containing 40% flufenacet and 20% diflufenican

FFA+ DFF SC600 containing 400 g/L flufenacet and 200 g/L diflufenican

FFA+FLT+DFF SC 360 containing 120 g/L flufenacet, 120 g/L flurtamone and 120 g/L diflufenican

FFA+DFF WG 70 containing 35% flufenacet and 35% diflufenican

**Table 7.3.1.2-3: Compilation of individual residue levels for flufenacet in supplementary trials**

Report No.		Application rate Flufenacet (g as/ha)	Residue levels grain (mg/kg)	Residue levels straw (mg/kg)
<b>Northern Europe</b>				
RA-2010/94		240	<0.05/ <0.05/ <0.05 /<0.05	<0.1/ <0.1/ <0.1/ <0.1
RA-2144/00		240	<0.05/ <0.05	<0.1/ <0.1
			<b>STMR &lt; 0.05</b>	<b>STMR &lt;0.1</b>
11-2095		110-120	<0.01/ 0.022	<0.05/ <0.05
11-2094		120	<0.01/ 0.017	<0.05/ <0.05
12-2001		120	<0.01/ <0.01/ <0.01/ <0.01	<0.05/ <0.05/ <0.05/ <0.05
			<b>STMR &lt;0.01</b>	<b>STMR &lt;0.05</b>
<b>Southern Europe</b>				
RA-2144/00		240-254	<0.05/< 0.05	< 0.1/ 0.11
09-2048		240	<0.01/ <0.01/ <0.01	< 0.05/ 0.06/ 0.06
09-2052		220-240	<0.01/ <0.01/ 0.01/ 0.05	<0.05/ <0.05/ <0.05/ 0.09
			<b>STMR &lt;0.01</b>	<b>STMR 0.06</b>
11-2095		120	0.02/ 0.035	<0.05/ <0.05
11-2094		120	<0.01/ <0.01	<0.05/ 0.059
12-2002		120	<0.01/ <0.01/ <0.01	<0.05/ <0.05/ 0.069
RA-2153/97		126	<0.05/ <0.05/ < 0.05/	<0.05/ <0.05/ < 0.05/
RA-2185/98		126	<0.05/ <0.05	<0.05/ <0.05
			<b>STMR 0.028</b>	<b>STMR &lt;0.05</b>

**B.7.3.1.2.1 Supplementary field trials – northern Europe (application rate 240 g as/ha)**

<b>Report:</b>	<b>KCA 6.3.1/03</b> , Seym, M.; 1996; M-004451-01-2
Title:	Determination of residues of FOE 5043 & Diflufenican 60 WG in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany
Document No & Report No:	M-004451-01-2 RA-2010/94 dated 1996-03-25
Guidelines:	Not indicated, fulfils EU 7029/VI/95 rev.5 dated 22 July 1997
GLP	Yes; Deviations: none

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**Material and methods**

Four trials on winter cereals (1 trial on barley, 1 trial on winter rye, and 2 trials on winter wheat) were conducted during the 1994-1995 growing season in Germany using a WG formulation containing 20% diflufenican + 40% flufenacet. The plants were treated in autumn (November), at growth stages ranging from BBCH 13 (3 leaves unfolded) to BBCH 25 (5 tillers detectable). The application rate was 240 g flufenacet/ha.

Green plant samples were taken for analysis at the growth stages BBCH 29 (end of tillering) and BBCH 51 (beginning of heading). Grain and straw samples were taken at normal harvest, which was between 246 and 253 days after application.

All samples were analysed for residues of flufenacet according to the method 00346 (Seym, M.; 1995; M-018864-02) which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. The method was reported in the original Annex II dossier (point 4). The procedure involves oxidation of the residues with potassium permanganate, hydrolysis with sulfuric acid, steam distillation, liquid/liquid partitioning, derivatisation with trifluoroacetic anhydride and GC/MS determination of the thus obtained 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (trifluoroacetamide). Residues are expressed as parent flufenacet.

Before analysis the samples were stored frozen for less than 8 months (237 days) for green material and less than 4 months (112 days) for grain and straw. These storage periods are adequately covered by the storage stability data for flufenacet.

**Findings**

Recovery rates were determined prior to analysis in order to validate the analytical method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide. The recovery rates and corresponding relative standard deviations (RSD) were satisfactory as shown in Table 7.3.1.2.1-1. The limit of quantification was 0.05 mg/kg in green plant and grain, and 0.10 mg/kg in straw. The residues in the barley, wheat, and rye samples from the individual trials are summarised in Table 7.3.1.2.1-2.

No apparent residues were found in any of the untreated samples, i.e. residues were < LOQ for flufenacet.

Flufenacet residues ranged between < 0.05 mg/kg to 0.1 mg/kg in green material collected at growth stage BBCH 29, while at the later growth stage (BBCH 51) residues have declined below the LOQ (0.05 mg/kg). In all trials, residues in grain and straw were below the LOQ of 0.05 mg/kg and 0.1 mg/kg, respectively.

**Table 7.3.1.2.1-1: Procedural recovery data for Flufenacet** (the LOQ is marked in bold).

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./meta-bolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2010/94 40044/0 0044-94  GLP: yes 1994	Barley, winter	green material	total residue flufenacet	3	<b>0.05</b>	84; 85; 91	84	91	87	4.4
				3	overall mg/kg		84	91	87	4.4
		grain	total residue flufenacet	3	<b>0.05</b>	81; 85; 91	81	91	86	5.9
				3	overall mg/kg		81	91	86	5.9
		straw	total residue flufenacet	3	<b>0.1</b>	80; 90; 92	80	92	87	7.4
				3	overall mg/kg		80	92	87	7.4
RA-2010/94 40045/9 0045-94  GLP: yes 1994	Rye, winter	green material	total residue flufenacet	12	<b>0.05</b>	79; 84; 87; 87; 90; 94; 94; 94; 94; 95; 96; 97	79	97	91	6.1
				8	0.5	81; 82; 86; 88; 92; 93; 93; 93	81	93	89	5.7
				20	overall mg/kg		79	97	90	6.0
		grain	total residue flufenacet	12	<b>0.05</b>	76; 79; 86; 86; 88; 89; 89; 90; 95; 96; 101; 105	76	105	90	9.2
				8	0.5	71; 79; 82; 84; 87; 90; 93; 94	71	94	85	9.1
				20	overall mg/kg		71	105	88	9.4
		straw	total residue flufenacet	12	<b>0.1</b>	79; 81; 82; 85; 85; 87; 88; 90; 90; 91; 93; 95	79	95	87	5.7
				8	1.0	69; 79; 79; 80; 81; 85; 87; 88	69	88	81	7.4
				20	overall mg/kg		69	95	85	7.2
RA-2010/94 40046/7 0046-94 and 40047/5 0047-94  GLP: yes 1994	Wheat, winter	green material	total residue flufenacet	6	<b>0.05</b>	71; 81; 84; 88; 95; 97	71	97	86	11.2
				6	overall mg/kg		71	97	86	11.2
		grain	total residue flufenacet	4	<b>0.05</b>	81; 91; 99; 109	81	109	95	12.5
				4	overall mg/kg		81	109	95	12.5
		straw	total residue flufenacet	3	<b>0.10</b>	79; 81; 94	79	94	85	9.6
				3	overall mg/kg		79	94	85	9.6

**Table 7.3.1.2.1-2: Residues of flufenacet in barley, wheat and rye after post-emergence application of flufenacet + diflufenican WG 60 (containing 40% flufenacet + 20% diflufenican) in northern Europe**

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2010/94 40044/0 0044-94 GLP yes 1994	Barley, winter Loreley	Germany Versuchsgut Höfchen, 51399 Burscheid Europe, North	60 WG	1	0.24	0.08	13	green material grain straw	124 201 253 253	<0.05 <0.05 <0.05 <0.1
RA-2010/94 40045/9 0045-94 GLP yes 1994	Rye, winter Gambit	Germany Versuchsgut Laacherhof, D-40789 Monheim Europe, North	60 WG	1	0.24	0.08	21	green material grain straw	94 172 246 246	0.06 <0.05 <0.05 <0.1
RA-2010/94 40046/7 0046-94 GLP yes 1994	Wheat, winter Contra	Germany Versuchsgut Höfchen, 51399 Burscheid Europe, North	60 WG	1	0.24	0.08	25	green material grain straw	119 191 247 247	<0.05 <0.05 <0.05 <0.10
RA-2010/94 40047/5 0047-94 GLP yes 1994	Wheat, winter Contra	Germany Versuchsgut Laacherhof, 40789 Monheim Europe, North	60 WG	1	0.24	0.08	21	green material grain straw	133 190 246 246	0.10 <0.05 <0.05 <0.1

\*Residues for total residue flufenacet determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet

DALT : Days after last treatment

## Conclusion

Four trials on winter cereals (1 trial on barley, 1 trial on winter rye, and 2 trials on winter wheat) were conducted during the 1994-1995 growing season in Germany to investigate the residues of flufenacet in cereals after application of 240 g flufenacet/ha and 120 g diflufenican/ha using a mixed WG formulation of the two substances. The plants were treated in autumn (November), at growth stages ranging from BBCH 13 (3 leaves unfolded) to BBCH 25 (5 tillers detectable). At mature harvest, the residues of flufenacet were < 0.05 mg/kg in grain and < 0.10 mg/kg in straw.

### B.7.3.1.2.2 Supplementary field trials – northern and southern Europe (application rate 240- 254 g as/ha)

<b>Report:</b>	<b>KCA 6.3.1/06, Hoffmann, M.; 2002; M-058156-01</b>
<b>Title:</b>	Determination of residues of FOE 5043 in/on wheat and barley following spray application of FOE 5043 & Diflufenican (600 SC) to winter wheat and winter barley in the field in Northern and Southern France, Germany and Spain
<b>Document No &amp; Report No:</b>	M-058156-01-1 RA-2144/00, dated 2002-04-12
<b>Guidelines:</b>	Directive 94/414/EEC Residues in or on treated products, food and feed EU 7029/VI/95 rev.5 dated 22 July 1997
<b>GLP</b>	Yes; Deviations: none

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**Material and methods**

Two trials on winter wheat and two trials on winter barley were conducted during the 2000-2001 growing season in northern and southern France, Germany and Spain using 'Flufenacet + Diflufenican SC 600'. The plants were treated at the growth stage BBCH 13 (3 leaves unfolded), which was usually in autumn (October - December), except in the Spanish trial, in which treatment was in February. The application rate was 240 g flufenacet/ha, except in the Spanish trial, in which the applied rate was slightly higher (254 g flufenacet/ha).

Grain and straw samples were taken at normal harvest, which was between 148 and 254 days after application.

All the samples were analysed for residues of flufenacet according to the method 00346 (Seym, M.; 1995; M-018864-02) which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis the grain and straw samples were stored frozen for less than 8 months (226 days). This storage period is adequately covered by the available storage stability data for flufenacet.

**Findings**

Recovery rates were determined prior to analysis in order to validate the analytical method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Fortification was performed by spiking control samples with one of the following compounds or a mixture thereof : parent flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide. The recovery rates and corresponding relative standard deviations (RSD) were satisfactory as shown in Table 7.3.1.2.2-1. The limit of quantification was 0.05 mg/kg in grain and 0.10 mg/kg in straw.

No residues were found in any of the untreated samples, i.e. residues were < LOQ for flufenacet.

The residues found in wheat and barley samples from the individual trials were below the LOQ for grain. Residues in straw were less than the LOQ in 3 trials and 0.11 mg/kg in the Spanish trial. The findings are summarised in Tables 7.3.1.2.2-2 (northern Europe) and 7.3.1.2.2-3 (southern Europe).

**Table 7.3.1.2.2-1: Procedural recovery data for Flufenacet** (the LOQ is marked in bold).

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2144/00  R 2000 0566/0 0566-00  and  R 2000 0567/9 0567-00  GLP: yes 2000	Wheat, winter	grain	total residue flufenacet	11	<b>0.05</b>	94; 84; 81; 98; 98; 91; 81; 77; 80; 80; 79	77	98	86	9.3
				1 12	0.5 overall mg/kg	73	73 73	73 98	73 85	10.0
		straw	total residue flufenacet	11	<b>0.10</b>	101; 93; 79; 88; 90; 92; 84; 81; 77; 83; 81	77	101	86	8.4
				1 12	1.0 overall mg/kg	75	75 75	75 101	75 85	8.9
RA-2144/00  R 2000 0568/7 0568-00  and  R 2000 0570/9 0570-00  GLP: yes 2000	Barley, winter	grain	total residue flufenacet	11	<b>0.05</b>	111; 104; 89; 84; 89; 73; 74; 73; 72; 77; 81	72	111	84	15.6
				1 12	0.5 overall mg/kg	80	80 72	80 111	80 84	15.0
		straw	total residue flufenacet	11	<b>0.10</b>	86; 83; 81; 79; 82; 74; 85; 81; 78; 95; 97	74	97	84	8.3
				1 12	1.0 overall mg/kg	81	81 74	81 97	81 84	8.0

Fortified with flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide or a mixture thereof; determined as FOE 5043 trifluoroacetamide and calculated as flufenacet equivalent

**Table 7.3.1.2.2-2: Residues of flufenacet in barley and wheat after post-emergence application of Flufenacet + Diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in northern Europe**

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2144/00 R 2000 0566 0 0566-00 GLP yes 2000	Wheat, winter Isen- grain	France F-37310 Chambourg sur Indre Europe, North	600 SC	1	0.24	0.08	13	grain	243	<0.05
								straw	243	<0.10
RA-2144/00 R 2000 0568 7 0568-00 GLP yes 2000	Barley, winter Theresa	Germany D-51399 Burscheid, Versuchs- gut Höfchen Europe, North	600 SC	1	0.24	0.08	13	grain	254	<0.05
								straw	254	<0.10

\*Residues for total residue flufenacet (determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet)  
 DALT : Days after last treatment

**Table 7.3.1.2.2-3: Residues of flufenacet in barley and wheat after post-emergence application of Flufenacet + Diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in southern Europe**

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2144/00 R 2000 0567 9 0567-00 GLP yes 2000	Wheat, winter Soissons	France F-31620 Gargas Europe, South	600 SC	1	0.24	0.08	13	grain	196	<0.05
								straw	196	<0.10
RA-2144/00 R 2000 0570 9 0570-00 GLP yes 2001	Barley, winter Graphic	Spain E-08289 Veciana Europe, South	600 SC	1	0.254	0.08	13	grain	148	<0.05
								straw	148	0.11

\*Residues for total residue flufenacet (determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet)  
 DALT : Days after last treatment

### Conclusion

Three trials on cereals (2 trials on wheat and 1 trial on barley) were conducted during the 2000-2001 growing season in northern and southern France, and in Germany using the 'Flufenacet + Diflufenican SC 600' formulation. The plants were treated in autumn at the growth stage BBCH 13 (3 leaves unfolded). Following application of 240 g flufenacet/ha, the residues of flufenacet at harvest were < 0.05 mg/kg in grain and < 0.10 mg/kg in straw.

A fourth trial with the 'Flufenacet + Diflufenican SC 600' formulation was performed on barley in Spain during the 2001 growing season. The plants were treated in February at the growth stage BBCH 13 (3 leaves unfolded). The application rate slightly exceeded the target rate at 254 g flufenacet/ha. At harvest, the residues of flufenacet were < 0.05 mg/kg in grain and 0.11 mg/kg in straw.

**B.7.3.1.2.3 Supplementary field trials – southern Europe (application rate 220 - 240 g as/ha)**

<b>Report:</b>	<b>KCA 6.3.1/07</b> , Billian, P.; Krusell, L.; 2010; M-361495-01_
Title:	Determination of the residues of diflufenican and flufenacet in/on winter barley after spraying of Flufenacet & Diflufenican SC 600 in the field in France (South)
Document No & Report No:	M-361495-01-1 09-2048 dated 2010-01-12
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed EU 7029/VI/95 rev.5 dated 22 July 1997
GLP	Yes; Deviations: none

**Material and methods**

Three trials on barley were conducted during the 2008-2009 growing season in southern France using the formulation 'Flufenacet + Diflufenican SC 600'. The plants were treated at the growth stage BBCH 13 (3 leaves unfolded) in late autumn (December). The application rate was 240 g flufenacet/ha.

Green plant samples were taken for analysis at the growth stage BBCH 13 immediately after application. Grain and straw samples were taken at normal harvest, which was between 188 and 203 days after application.

All the samples were analysed for residues of flufenacet according to the method 01179 (Class, Th.; Merdian, H.; 2010; M-362716-01), which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. The procedure involves oxidation of the residues with potassium permanganate, hydrolysis with sulfuric acid, steam distillation, liquid/liquid partitioning, and LC-MS/MS determination of the thus obtained 4-fluoro-*N*-isopropylaniline. Residues are expressed as parent flufenacet.

Before analysis, samples were stored frozen for less than 11 months for green material and 4 months for grain and straw (329 days for green material, 113 days for grain and 115 days for straw). These storage periods are adequately covered by the storage stability data for flufenacet.

**Findings**

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The overall mean recoveries for total residue of flufenacet were within the acceptable range of 70 – 110% (RSD < 20%) with the exception of green material when fortified with flufenacet and for straw when fortified with the mixture of the metabolites where values were just below guideline requirements (67 and 69% respectively), see Table 7.3.1.2.3-1.

The limit of quantification was 0.01 mg/kg in grain and green material, and 0.05 mg/kg in straw.

No apparent residues were found in any of the untreated samples, i.e. residues were < LOQ for flufenacet.

Flufenacet derived residues in grain were < 0.01 mg/kg and ranged from < 0.05 – 0.06 mg/kg in straw. The residues found in the barley samples from the individual trials are summarised in Table 7.3.1.2.3-2.

**Table 7.3.1.2.3-1: Procedural recoveries for flufenacet in winter barley** (the LOQ is marked in bold).

Study Trial No. Plot No.					Fortific ation level (mg/kg)	Recovery (%)				
GLP Year	Crop	Portion analysed	a.s./metabolite	n		Individual recoveries	Min	Max	Mean	RSD
Fortified with flufenacet										
09-2048	Barley, winter	green material	total residue flufenacet	4	<b>0.01</b>	66;68;74;67	66	74	69	5.2
09-2048-01				1	5.0	60	60	60		
09-2048-02				1	12	66	66	66		
09-2048-03				6	overall		60	74	67	6.7
GLP: yes 2008		grain	total residue flufenacet	3	<b>0.01</b>	92;79;62	62	92	78	19.4
				4	0.10	71;65;69;80	65	80	71	8.9
				7	overall		62	92	74	14.0
				straw	total residue flufenacet	2	<b>0.05</b>	87;87	87	87
		2	0.50			62;62	62	62	62	
		4	overall				62	87	75	19.4
Fortified with mixture of flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide (1/1/1)										
09-2048	Barley, winter	green material	total residue flufenacet	1	<b>0.01</b>	116	-	-	-	-
09-2048-01				1	2.4	90	-	-	-	
09-2048-02				2	overall		90	116	103	-
09-2048-03		grain	total residue flufenacet	1	<b>0.01</b>	83	-	-	-	-
GLP: yes 2008				1	0.10	67	-	-	-	-
				2	overall		67	83	75	-
		straw	total residue flufenacet	2	<b>0.05</b>	71, 67	67	71	69	
				2	overall		67	71	69	-

**Table 7.3.1.2.3-2: Residues of flufenacet in barley after post-emergence application of flufenacet + diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in southern Europe**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
09-2048 09-2048-01 GLP: yes 2008	Barley, winter Platine	France 31620 Castelnau d'Estretfonds Europe, South	600 SC	1	0.24	0.080	13	green material grain straw	0 197 197	9.2 <0.01 <0.05
09-2048 09-2048-02 GLP: yes 2008	Barley, winter Baraka	France 84490 St Saturnin les Apt Europe, South	600 SC	1	0.24	0.080	13	green material grain straw	0 188 188	11 <0.01 0.06
09-2048 09-2048-03 GLP: yes 2008	Barley, winter Esterel	France 86170 Ibeil Europe, South	600 SC	1	0.24	0.080	13	green material grain straw	0 203 203	9.5 <0.01 0.06

\*Residues for total residue flufenacet (determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet)  
DALT : Days after last treatment

### Conclusion

Three trials on barley were conducted in the southern part of France during the 2008/2009 growing season. The product 'Flufenacet + Diflufenican SC 600' was applied at a rate of 0.6 L/ha corresponding to 240 g flufenacet/ha and 120 kg diflufenican/ha. Treatment was performed in autumn at the growth stage BBCH 13. At mature harvest flufenacet residues were < 0.01 mg/kg in grain and < 0.05-0.06 mg/kg in straw.

<b>Report:</b>	<b>KCA 6.3.1/08, Billian, P.; et al.; 2010; M-363200-02</b>
<b>Title:</b>	Determination of the residues of diflufenican and flufenacet in/on winter wheat after spraying of Flufenacet & Diflufenican SC 600 in the field in France (south)
<b>Document No &amp; Report No:</b>	M-363200-02-1 09-2052 dated 2010-08-05
<b>Guidelines:</b>	Directive 94/414/EEC Residues in or on treated products, food and feed EU 7029/VI/95 rev.5 dated 22 July 1997
<b>GLP</b>	yes

### Material and methods

Four trials on winter wheat were conducted during the 2008/2009 growing season in southern France using a SC formulation containing 200 g/L diflufenican and 400 g/L flufenacet. The plants were treated in late autumn and winter (December-January), at growth stages ranging from BBCH 13 (3 leaves unfolded) to BBCH 21 (first tiller detectable). The application rate was 240 g flufenacet /ha in 3 trials. In one trial the application rate was underdosed by 7% (220 g flufenacet /ha).

Green plant samples were taken for analysis at the growth stages BBCH 13 immediately after application.

Grain and straw samples were taken at normal harvest, which was between 153 and 220 days after application.

All samples were analysed for residues of flufenacet according to the method 01179 (Class, Th.; Meridian, H.; 2010; M-362716-01) , which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen for less than 12 months (352 days) for green material and about 5 months (155 and 143 days) for grain and straw, respectively.

All storage periods are adequately covered by the storage stability data for flufenacet.

### Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. For flufenacet fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding RSD were satisfactory (cf. Table 7.3.1.2.3-3). The limit of quantification was 0.01 mg/kg in green plant and grain, and 0.05 mg/kg in straw.

No residues were found in any of the untreated samples. Flufenacet derived residues in grain ranged from < 0.01-0.05 mg/kg. In straw residues were < 0.05 mg/kg in 3 trials and 0.09 mg/kg in one trial.

The residues found in the wheat samples from the individual trials are summarised in Tables 7.3.1.2.3-4.

**Table 7.3.1.2.3-3: Procedural recoveries for flufenacet in winter wheat**

The LOQ is marked in bold.

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
Fortified with flufenacet										
09-2052  09-2052-01 09-2052-02 09-2052-03 09-2052-04  GLP: yes 2008	Wheat, winter	green material	total residue flufenacet	1	0.10	74	74	74	74	
				1	20	75	75	75	75	
				3	30	82; 87; 95	82	95	88	7.5
				5	overall		74	95	83	10.6
		grain	total residue flufenacet	1	<b>0.01</b>	97	97	97	97	
				2	0.10	103;91	91	103	97	
				3	overall		91	103	97	6.2
		straw	total residue flufenacet	1	<b>0.05</b>	70	70	70	70	
				2	0.50	87;84	84	87	86	
				3	overall		70	87	80	11.3
Fortified with mixture of flufenacet oxalate hydrate/flufenacet sulfonic acid sodium salt/flufenacet thioglycolate sulfoxide (1/1/1)										
09-2052  09-2052-01 09-2052-02 09-2052-03 09-2052-04  GLP: yes 2008	Wheat, winter	green material	total residue flufenacet	1	<b>0.01</b>	69	-	-	-	-
				2	18	72, 73	72	73	73	-
				1	27	66	-	-	-	-
				2	30	67, 65	65	67	66	-
				6	overall		65	73	69	4.8
		grain	total residue flufenacet	3	<b>0.01</b>	83, 73, 72	72	83	76	8.0
				3	0.10	79, 89, 77	77	89	82	7.9
				6	overall		72	89	79	8.1
		straw	total residue flufenacet	1	<b>0.05</b>	71	-	-	-	-
				4	0.6	69, 74, 85, 92	69	92	80	13
				5	overall		69	92	78	13

**Table 7.3.1.2.3-4: Residues of flufenacet in wheat after post-emergence application of flufenacet + diflufenican SC 600 (containing 400 g/L flufenacet + 200 g/L diflufenican) in southern Europe**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
09-2052 09-2052-01 GLP: yes 2008	Wheat, winter Arlequin	France 47550 Boe Europe, South	600 SC	1	0.24	0.080	13	green material grain straw	0 209 209	17 0.01 <0.05
09-2052 09-2052-02 GLP: yes 2009	Wheat, winter Aubusson	France 26300 Alixan Europe, South	600 SC	1	0.24	0.080	21	green material grain straw	0 153 153	22 <0.01 <0.05
09-2052 09-2052-03 GLP: yes 2008	Wheat, winter Aubusson	France 69650 Quincieux Europe, South	600 SC	1	0.22	0.085	13	green material grain straw	0 196 196	17 0.05 0.09
09-2052 09-2052-04 GLP: yes 2008	Wheat, winter Mendel	France 79120 Lezay Europe, South	600 SC	1	0.24	0.080	13	green material grain straw	0 220 220	24 <0.01 <0.05

\*Residues for total residue flufenacet (determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet)  
 DALT : Days after last treatment

## Conclusion

Four trials on winter wheat were performed during the 2008/2009 growing season in southern France to investigate the residues of flufenacet (and diflufenican) in cereals after application of 240 g flufenacet/ha (and 120 g diflufenican /ha) using a mixed SC formulation of the two substances. The plants were treated in autumn or winter in case of wet weather conditions (December-January), at growth stages BBCH 13 (3 leaves unfolded) to BBCH 21 (first tiller detectable). At mature harvest, the residues of flufenacet amounted to < 0.01-0.05 mg/kg in grain and <0.05-0.09 mg/kg in straw.

### Summary conclusion for the use of flufenacet in northern and southern Europe with use patterns involving 240 g flufenacet/ha (B.7.3.1.2.1 – B.7.3.1.2.3)

The set of residue data on wheat, barley and rye conducted with the straight formulation WG 60 and evaluated for Annex I inclusion is considered appropriate to also support the representative use for the mixed product 'flufenacet + diflufenican SC 600' at a rate of 240 g flufenacet/ha in northern Europe. The use pattern for both products involve the same application parameters and residue data obtained from trials using a WG formulation are considered appropriate to also support SC formulations. Both formulations types are known to produce comparable residues, particularly if the application is conducted early during the crop development. In all trials, residues have shown to be less than the LOQ for grain (< 0.05 mg/kg) and straw (< 0.1 mg/kg).

Nevertheless, 6 trials on wheat, barley and rye are reported for the northern region with WG and SC formulations at an application rate of 240 g flufenacet/ha which demonstrate that the residue behaviour of flufenacet does not alter when applied in a mixture with diflufenican. Applications were performed early post-emergence during leaf development until mid of tillering (BBCH 13-25). Residues in grain and straw were always below the LOQ of 0.05 or 0.1 mg/kg, respectively.

No residue data for flufenacet from the southern region were evaluated for Annex I inclusion. With the present dossier 9 trials are submitted to support the use pattern at 240 g as/ha with early post-emergence application. The trials were already evaluated at a national level (evaluating member state France, product name FOSBURI). Flufenacet was applied at rates ranging from 220 – 254 g as/ha during leaf development until beginning of tillering (BBCH 13-21). The trials on wheat and barley were conducted over two growing seasons. Residues in grain ranged from < 0.01 to 0.05 mg/kg (median < 0.01 mg/kg), and in straw from 0.05 to 0.11 mg/kg (median 0.06 mg/kg).

The data sets from the northern and southern region are considered to represent the critical GAPs for flufenacet.

The data sets were recently reviewed by the RMS France and EFSA and the data set from southern Europe forms the basis for the new MRL value of 0.1 mg/kg as established in Commission Regulation (EU) No 1127/2014.

#### B.7.3.1.2.4 Supplementary field trials in northern Europe (application rate 120 g as/ha)

<b>Report:</b>	<b>KCA 6.3.1/10</b> , Stuke, S.; Ballmann, C.; 2013; M-459795-01_
<b>Title:</b>	Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after Spraying of DFF & FFA & FLT SC 360 in the field in Germany, Belgium and the Netherlands
<b>Document No</b> <b>Report No</b>	M-459795-01-1 Study no. 12-2001 dated 2013-07-09
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>Regulation (EC) no 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC</li> <li>EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997)</li> <li>OECD 509 Adopted 2009-09-07; Crop Field Trial</li> <li>US EPA OCSPP Guideline No. 860.1500</li> </ul>
<b>GLP</b>	Yes ; Deviations : none

#### Material and methods

Four trials on winter wheat (2) and winter barley (2) were conducted during the 2011/2012 growing season in Germany (2), Belgium (1) and the Netherlands (1) using an SC formulation containing 120 g/L flufenacet, 120 g/L diflufenican and 120 g/L flurtamone (DFF+FFA+FLT SC 360). The plants were treated in late autumn (November) in 3 trials, at growth stage BBCH 22/23. In one trial the requested growth stage was not reached in autumn and thus the application was conducted in spring (April) at BBCH 25. The application was at the required rate in all trials (120 g flufenacet /ha).

Green plant samples were taken for analysis at the growth stages BBCH 49 (forage stage) and at BBCH 83 (silage stage, whole plant without root). Grain and straw samples were taken at normal harvest, which was between 112 and 263 days after application.

All samples were analysed for residues of flufenacet according to the method 01100/M002 (Stuke, S.; Teubner, L.; 2013; M-448503-01 ), which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen up to 12 months (371 days) for green material/whole plants without root and up to 10 months (300 days) for grain and straw.

All storage periods are adequately covered by the storage stability data for flufenacet.

### Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. The recovery-rates and relative standard deviations (RSD) were satisfactory (cf. Table 7.3.1.2.4-1). The limit of quantification was 0.01 mg/kg in green plant material and grain, and 0.05 mg/kg in straw.

The residues of flufenacet in the untreated samples were < LOQ. The residues found in the wheat and barley samples from the individual trials are summarised in Table 7.3.1.2.4-2. Flufenacet derived residues in green material at forage stage and silage stage, in grain and straw were less than the LOQ (< 0.01 mg/kg for green plant material and grain and < 0.05 mg/kg for straw) in all trials.

**Table 7.3.1.2.4-1: Procedural recovery data for Flufenacet (the LOQ is marked in bold).**

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
12-2001  12-2001-01 12-2001-02 12-2001-03 12-2001-04  GLP: yes 2011	Barley, winter	green material <sup>#</sup>	total residue flufenacet	6	<b>0.01</b>	77;82;96;98;103;105	77	105	94	12.2
				4	0.10	84;88;90;104	84	104	92	9.5
				3	1.0	91;92;102	91	102	95	6.4
				1	20	77	77	77	77	
				14	overall		77	105	92	10.5
		grain	total residue flufenacet	4	<b>0.01</b>	69;79;94;95	69	95	84	14.9
				2	0.10	89;91	89	91	90	
				6	overall		69	95	86	11.8
		straw	total residue flufenacet	2	<b>0.05</b>	88;97	88	97	93	
				2	0.50	94;97	94	97	96	
				4	overall		88	97	94	4.5

<sup>#</sup> Sample materials green material and whole plant without root are grouped to the sample group cereals green material.

Fortified with flufenacet, determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet

The recoveries were performed during the conduct of the study 12-2001, 12-2002 (and 12-2003, not reported).

**Table 7.3.1.2.4-2: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in northern Europe**

Study Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Growth stage (BBCH)	total residue flufenacet* (mg/kg)
12-2001 12-2001-01 GLP: yes 2011	Barley, winter Meridian	Germany 49377 Langförden Europe, North	360 SC	1	0.12	0.040	23	green material	170	49	<0.01
								whole plant	216	83	<0.01
								without roots	244	92	<0.01
								grain straw	244	92	<0.05
12-2001 12-2001-02 GLP: yes 2011	Barley, winter Saskia (early 6-rows variety, mid height)	Belgium 6210 Villers-Perwin Europe, North	360 SC	1	0.12	0.040	22	green material	181	49	<0.01
								whole plant	209	83	<0.01
								without roots	252	89	<0.01
								grain straw	252	89	<0.05
12-2001 12-2001-03 GLP: yes 2011	Wheat, winter Inspiration	Germany 59457 Werl-Westönnen Europe, North	360 SC	1	0.12	0.040	22	green material	192	49	<0.01
								whole plant	239	83	<0.01
								without roots	263	89	<0.01
								grain straw	263	89	<0.05
12-2001 12-2001-04 GLP: yes 2012	Wheat, winter Taureq winter	Netherlands 1774 PE Slootdorp Europe, North	360 SC	1	0.12	0.040	25	green material	41	49	<0.01
								whole plant	83	83	<0.01
								without roots	112	89	<0.01
								grain straw	112	89	<0.05

\*Residues for total residue flufenacet (determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet)

DALT : Days after last treatment

## Conclusion

Four trials on winter wheat and winter barley were performed during the 2011/2012 growing season in northern Europe to investigate the residues of flufenacet (and flurtamone) in cereals after application of 120 g flufenacet/ha using a triple mixture also containing flurtamone and diflufenican. The plants were treated in autumn at growth stages BBCH 22/23 or in spring in one trial in case of delayed development (BBCH 25). Residues of flufenacet were < 0.01 mg/kg in green material at forage and silage stage. At mature harvest, flufenacet derived residues were < 0.01 mg/kg in grain and <0.05 mg/kg in straw.

**B.7.3.1.2.5 Supplementary field trials in northern and southern Europe (application rate 110-120 g as/ha)**

<b>Report:</b>	<b>KCA 6.3.1/12</b> , Noss, G.; van Berkum, S.; 2013; M-460003-01_
<b>Title:</b>	Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter barley after spray application of DFF & FFA & FLT SC 360 in Germany, the United Kingdom, southern France and Italy
<b>Document No Report No</b>	M-460003-01-1 Study No. 11-2094 dated 2013-07-11
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>• Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC</li> <li>• OECD 509 Adopted 2009-09-07, Crop Field Trial</li> <li>• EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)</li> <li>• US EPA OCSPP Guideline No. 860.1500</li> </ul>
<b>GLP</b>	Yes ; Deviations : none

<b>Report:</b>	<b>KCA 6.3.1/09</b> , Noss, G.; Diehl, P.; 2013; M-459755-01_
<b>Title:</b>	Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter wheat after spray application of DFF & FFA & FLT SC 360 in Germany, the Netherlands, southern France and Spain
<b>Document No Report No</b>	M-459755-01-1 Study No. 11-2095 dated 2013-07-10
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>• Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC</li> <li>• OECD 509 Adopted 2009-09-07, Crop Field Trial</li> <li>• EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)</li> <li>• US EPA OCSPP Guideline No. 860.1500</li> </ul>
<b>GLP</b>	Yes ; Deviations : none

**Material and methods**

In total 8 trials on winter wheat and winter barley were conducted during the 2010/2011 growing season. Four trials were conducted in the northern European climatic zone (2 trials on barley, 2 trials on wheat) in Germany (2), the Netherlands (1) and the United Kingdom (1). Four trials (2 trials on barley, 2 trials on wheat) were conducted in the southern European climatic zone: France (2), Spain (1) and Italy (1). For all trials an SC formulation containing 120 g/L flufenacet, 120 g/L diflufenican and 120 g/L flurtamone (DFF+FFA+FLT SC 360) has been used.

The application schedule called for application of 1 L product/ha (corresponding to 120 g flufenacet/ha) at growth stage BBCH 25. In the northern zone, the plants were treated between January and April at growth stage BBCH 25. Due to extreme dry weather conditions, in one trial delayed germination resulted in a range of growth stages at application (actual BBCH 23 to 27), however, the average was estimated to be at BBCH 25.

In the southern zone plants were treated in March at growth stage BBCH 25 to 30. Due to unfavourable weather conditions the treatment was slightly delayed in 3 trials.

The application was at the required rate (120 g flufenacet /ha) in all trials except one from the northern zone where the application rate was slightly underdosed (110 g flufenacet /ha).

Samples of green material at early growth stages were taken to generate residue data needed to refine the ecotoxicological evaluation (day 0, 1, 3, 5, 14).

Green plant samples were taken for analysis at the growth stages BBCH 51 (forage stage) and at BBCH 83 (silage stage, whole plant without root).

Grain and straw samples were taken at normal harvest, which was between 117 and 262 days after application for the northern European trials and 80 and 119 days for the southern European trials.

All samples were analysed for residues of flufenacet according to the method 01100/M002 (Stuke, S.;

Teubner, L.; 2013; M-448503-01 ) which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen up to 25 months (738 and 748 days) in study 11-2094 and 11-2095. All storage periods are adequately covered by the storage stability data for flufenacet.

### Findings

The relative dry matter of control and treated samples of cereals green material harvested at forage stage (BBCH 51) and whole plant without root at silage stage (BBCH 83) was determined for studies 11-2094 and 11-2095. The determination of relative dry matter content was not conducted according to GLP. The results of the determination of relative dry matter for these samples are shown in Table 7.3.1.2.5-1.

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. The recovery-rates and relative standard deviations (RSD) were satisfactory (cf. Table 7.3.1.2.5-2). The limit of quantification was 0.01 mg/kg in green plant material and grain, and 0.05 mg/kg in straw.

No residues were found in the untreated samples, i.e. residues were < LOQ for flufenacet except for barley green material samples (for three trials) collected on the day of treatment where the residues ranged from 0.012 to 0.022 mg/kg and one exception at 0.016 mg/kg in barley green material sample at BBCH 51 (DALT 181). Residues found in control samples were identified as contaminations in the water steam distilleries originating from the high residues of flufenacet found in the treated samples of green material at day 0 - 6. Since analysis of green material at early growth stages (day 0 to 14) intended for ecotoxicological evaluations was not needed for flufenacet, it was decided not to re-analyze these samples. All apparatus was thoroughly cleaned and tested for any further analyses.

The residues found in the wheat and barley samples from the individual trials are summarised in Tables 7.3.1.2.5-3 (northern Europe) and 7.3.1.2.5-4 (southern Europe).

Northern Europe: Flufenacet derived residues in green material at forage stage (BBCH 51) ranged between < 0.01 and 0.077 mg/kg, and residues in whole plant without root (BBCH 83) were between <0.01 and 0.019 mg/kg. Residues in grain amounted to <0.01 – 0.022 mg/kg and were less than the LOQ (0.05 mg/kg) in straw.

Southern Europe: Residues at forage stage of green plant material were between 0.027 and 0.081 mg/kg and at silage stage between 0.017 and 0.061 mg/kg. In grain at harvest, residues ranged from <0.01 to 0.035 mg/kg and from <0.05 to 0.059 mg/kg in straw.

**Table 7.3.1.2.5-1: Relative dry matter content of control and treated samples at forage and silage stage**

Trial no. Country	Control (C) / Treated (T)	Growth stage [BBCH]	DALT	Crop	Sample material	Relative dry matter [%]
North European climatic zone						
11-2095-01 Germany	C	51	51	wheat	green material	21.9
	T	51	51		green material	24.9
	C	83	93		whole plant without root	41.9
	T	83	93		whole plant without root	39.1
11-2095-02 Netherlands	C	47-57*	43	wheat	green material	23.8
	T	47-57*	43		green material	22.3
	C	83	95		whole plant without root	43.6
	T	83	95		whole plant without root	43.1
11-2094-01 Germany	C	51	181	barley	green material	17.3
	T	51	181		green material	15.4
	C	83	209		whole plant without root	29.8
	T	83	209		whole plant without root	30.3
11-2094-02 United Kingdom	C	51	119	barley	green material	19.4
	T	51	119		green material	22.3
	C	83	164		whole plant without root	34.3
	T	83	164		whole plant without root	38.3
South European climatic zone						
11-2095-03 France	C	51	57	wheat	green material	21.9
	T	51	57		green material	21.3
	C	83	90		whole plant without root	33.3
	T	83	90		whole plant without root	33.6
11-2095-04 Spain	C	51	42	wheat	green material	30.3
	T	51	42		green material	25.8
	C	83	68		whole plant without root	43.6
	T	83	68		whole plant without root	44.8
11-2094-03 France	C	51	55	barley	green material	27.9
	T	51	55		green material	25.8
	C	83	83		whole plant without root	40.7
	T	85	83		whole plant without root	43.8
11-2094-04 Italy	C	51	28	barley	green material	23.1
	T	51	28		green material	22.7
	C	83	50		whole plant without root	30.6
	T	83	50		whole plant without root	30.6

\* Due to extreme dry weather conditions, germination was partly delayed resulting in a range of different growth stages at sampling.

**Table 7.3.1.2.5-2: Procedural recoveries for flufenacet in/on wheat and barley** (the LOQ is marked in bold).

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
11-2094 11-2094-01 to 11-2094-04  11-2095 11-2095-01 to 11-2095-04  GLP: yes 2011	Barley, Wheat	green material*	total residue flufenacet	3	<b>0.01</b>	106;109;115	106	115	110	4.2
				4	0.10	90;96;104;118	90	118	102	11.9
				1	1.0	79	79	79	79	
				1	10	85	85	85	85	
				1	20	77	77	77	77	
				10	overall		77	118	98	15.0
		grain	total residue flufenacet	3	<b>0.01</b>	83;91;114	83	114	96	16.8
				9	0.10	88;91;97;98;101;108;111;116;113	88	116	103	9.7
				1	1.0	116	116	116	116	
				1	10	76	76	76	76	
				2	20	75;96	75	96	86	
				16	overall		75	116	98	14.0
		straw	total residue flufenacet	1	<b>0.05</b>	78**	78	78	78	
				1	0.50	93**	93	93	93	
				2	overall		78	93	86	

RSD = Relative standard deviation, n = number of tests,

Fortified with flufenacet, determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet

\*Samples of green material and whole plant without root were combined to “green material” for calculation of the mean value and RSD.

\*\* These recoveries exclusively were conducted during the study 11-2094 in barley straw which is also representative for wheat straw.

**Table 7.3.1.2.5-3: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in northern Europe**

Study Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Growth stage (BBCH)	total residue flufenacet (mg/kg)
11-2094 11-2094-01 GLP: yes 2011	Barley, winter Ketos winter barley	Germany 51399 Burscheid Europe, North	360 SC	1	0.12	0.040	25	green material	0	25	3.3
									1	25	1.7
									3	25	1.6
									5	25	1.6
									14	29	1.0
									181	51	<0.01/0.016*
								whole plant without roots	209	83	0.019
								grain	262	89	0.017
								straw	262	89	<0.05
11-2094 11-2094-02 GLP: yes 2012	Barley, winter Carat Winter Barley	United Kingdom SG8 8SS Cambridge Europe, North	360 SC	1	0.12	0.060	25	green material	0	25	14/0.022*
									1	25	14
									3	25	2.5
									4	25	3.9
									14	26	1.8
									119	51	0.037
								whole plant without roots	164	83	<0.01
								grain	203	89	<0.01
								straw	203	89	<0.05
11-2095 11-2095-01 GLP: yes 2011	Wheat, winter Akteur	Germany 59457 Werl - Niederbergstrasse Europe, North	360 SC	1	0.12	0.040	25	green material	0	25	4.7/0.012*
									1	25	3.6
									3	26	2.5
									5	27	1.6
									14	31	0.42
									51	51	0.020
								whole plant without roots	93	83	0.015
								grain	117	89	0.022
								straw	117	89	<0.05
11-2095 11-2095-02 GLP: yes 2011	Wheat, winter Tabasco	Netherlands 1175 KD Lynden (Hoofddorp) Europe, North	360 SC	1	0.1104	0.0399	25-27	green material	0	25	16/0.022*
									1	25	12
									3	27	5.0
									5	26	3.8
									14	28	0.66
									43	57	0.077
								whole plant without roots	95	83	<0.01
								grain	121	89	<0.01
								straw	121	89	<0.05

Total residue flufenacet: Final determination as 4-fluoro-*N*-isopropylaniline, residues calculated as flufenacet.

\* Residues found in control samples were identified as contaminations in the water steam distilleries originating from the high residues of flufenacet found in the treated samples of green material at day 0-5. All apparatus was thoroughly cleaned and tested for any further analyses.

**Table 7.3.1.2.5-4: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in southern Europe**

Study Trial No. GLP Year	Crop Variety	Country	Application					Residues			
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Growth stage (BBCH)	total residue flufenacet (mg/kg)
11-2094 11-2094-03 GLP: yes 2011	Barley, winter Kétos Winter Barley	France 86220 Leugny Europe, South	360 SC	1	0.12	0.040	29	green material	0	29	5.2
									1	29	5.1
									3	29	3.3
									6	29	1.4
								whole plant without roots	14	30	0.48
									55	51	0.027
									83	85	0.031
								grain straw	108	89	<0.01
									108	89	<0.05
11-2094 11-2094-04 GLP: yes 2011	Barley, winter Aldebaran winter variety	Italy 44124 Ferrara Europe, South	360 SC	1	0.12	0.030	25	green material	0	25	3.0/0.024*
									1	25	2.9
									3	26	1.8
									5	26	1.2
								whole plant without roots	14	31	0.44
									28	51	0.081
									50	83	0.044
								grain straw	80	89	<0.01
									80	89	0.059
11-2095 11-2095-03 GLP: yes 2011	Wheat, winter Cezanne	France 86270 Mairé Europe, South	360 SC	1	0.12	0.040	29	green material	0	29	12/0.013*
									1	29	8.6
									3	29	4.3
									6	29	1.9
								whole plant without roots	14	30	0.70
									57	51	0.033
									90	83	0.017
								grain straw	119	89	0.020
									119	89	<0.05
11-2095 11-2095-04 GLP: yes 2011	Wheat, winter Moncada; sowing seed production	Spain 08520 Marata - Les Franqueses Europe, South	360 SC	1	0.12	0.040	30	green material	0	30	8.3/0.015*
									1	30	8.3
									2	30	5.1
									5	31	0.84
								whole plant without roots	14	32	0.41
									42	51	0.071
									68	83	0.061
								grain straw	103	89	<0.05
									103	89	<0.05

Total residue flufenacet: Final determination as 4-fluoro-N-isopropylaniline, residues calculated as flufenacet.

\* Residues found in control samples were identified as contaminations in the water steam distilleries originating from the high residues of flufenacet found in the treated samples of green material at day 0-6. All apparatus was thoroughly cleaned and tested for any further analyses.

**Conclusion:**

Four trials per geographical region on wheat and barley were performed during the 2010/2011 growing season (8 trials in total) to investigate the residues of flufenacet (as well as flurtamone and diflufenican) in cereals after application of 120 g flufenacet/ha (and 120 g flurtamone/ha, 120 g diflufenican/ha) using a triple mixture. The use pattern called for application at mid tillering, however, due to unfavourable weather conditions the application was slightly delayed up to BBCH 29/30 in 3 southern European trials but still within tillering stage.

**Northern Europe:** Flufenacet derived residues in green material at forage stage (BBCH 51) ranged between < 0.01 and 0.077 mg/kg, and residues in whole plant without root (BBCH 83) were between <0.01 and 0.019 mg/kg. Residues in grain amounted to <0.01 – 0.022 mg/kg and were less than the LOQ (0.05 mg/kg) in straw.

**Southern Europe:** Residues at forage stage of green material were between 0.027 and 0.081 mg/kg and at silage stage between 0.017 and 0.061 mg/kg. In grain at harvest, residues ranged between <0.01 and 0.035 mg/kg and were <0.05-0.059 mg/kg in straw.

The deviation to the rate of the supported GAP (160 g as/ha) is within the EU's tolerance criteria for comparability (-25%).

**B.7.3.1.2.6 Supplementary field trials in southern Europe (application rate flufenacet: 120 g as/ha)**

<b>Report:</b>	<b>KCA 6.3.1/11</b> , Stuke, S.; et al.; 2013; M-459799-01_
<b>Title:</b>	Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after spray application of DFF & FFA & FLT SC 360 in Southern France, Italy, Spain and Portugal
<b>Document No</b> <b>Report No</b>	M-459799-01-1 Study No. 12-2002 dated 2013-07-09
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC</li> <li>EC guidance working document 7029/VI/95 rev. 5 (July 22, 1997)</li> <li>OECD 509 Adopted 2009-09-07, OECD Guideline for the testing of chemicals;</li> <li>Crop Field Trial; US EPA OCSPP Guideline No. 860.1500</li> </ul>
<b>GLP</b>	Yes ; Deviations : none

**Material and methods**

Four trials on winter wheat (2) and winter barley (2) were conducted during the 2011/2012 growing season in southern France, Italy, Spain and Portugal using an SC formulation containing 120 g/L flufenacet, 120 g/L diflufenican and 120 g/L flurtamone (DFF+FFA+FLT SC 360). The application was at the required rate in all trials (120 g flufenacet /ha) except in one trial (03) where the dose rate was slightly less (7% of the target rate). Since the latter trial is out of the 25% range for comparability relative to the application rate of the supported GAP, this trial is disregarded in the following tables.

The plants in the remaining 3 trials were treated in late autumn (November, December) or beginning of March when the requested growth stage was not reached in autumn. Treatments were conducted at growth stage BBCH 22 to 25.

Green plant samples were taken for analysis at the growth stages BBCH 49 (forage stage) and at BBCH 83 (silage stage, whole plant without root). Grain and straw samples were taken at normal harvest, which was between 119 and 213 days after application.

All samples were analysed for residues of flufenacet according to the method 01100/M002 (Stuke, S.; Teubner, L.; 2013; M-448503-01) which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

Before analysis for flufenacet the samples were stored frozen up to 14 months (414 days) for green material/whole plants without root and up to 12 months (346 days) for grain and straw.

All storage periods are adequately covered by the storage stability data for flufenacet.

### Findings

Recovery rates were determined concurrently with the sample analysis in order to check the accuracy of the residue analysis. The recovery-rates and relative standard deviations (RSD) were satisfactory (cf. Table 7.3.1.2.6-1). The limit of quantification was 0.01 mg/kg in green plant material and grain, and 0.05 mg/kg in straw.

The residues of flufenacet in the untreated samples were < LOQ. The residues found in the barley and wheat samples from the individual trials are summarised in Table 7.1.3.2.6-2. Residues of flufenacet ranged between < 0.01 and 0.035 mg/kg in green plant material at forage stage and between < 0.01 and 0.045 mg/kg at silage stage (whole plant without root). At harvest, flufenacet derived residues in grain were < 0.01 mg/kg. In straw, residues amounted to < 0.05- 0.069 mg/kg.

**Table 7.3.1.2.6-1: Procedural recovery data for Flufenacet** (the LOQ is marked in bold).

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
12-2002	Barley, winter	green material <sup>#</sup>	total residue flufenacet	6	<b>0.01</b>	77;82;96;98;103; 105	77	105	94	12.2
12-2002-01				4	0.10	84;88;90;104	84	104	92	9.5
12-2002-02				3	1.0	91;92;102	91	102	95	6.4
12-2002-04				1	20	77	77	77	77	
				14	overall		77	105	92	10.5
yes 2011		grain	total residue flufenacet	4	<b>0.01</b>	69;79;94;95	69	95	84	14.9
				2	0.10	89;91	89	91	90	
				6	overall		69	95	86	11.8
		straw	total residue flufenacet	2	<b>0.05</b>	88;97	88	97	93	
				2	0.50	94;97	94	97	96	
				4	overall		88	97	94	4.5

<sup>#</sup> Sample materials green material and whole plant without root are grouped to the sample group cereals green material.

Fortified with flufenacet, determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet

Recoveries were performed during the conduct of the study 12-2001, 12-2002 (and 12-2003, not reported).

**Table 7.3.1.2.6-2: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican + flurtamone SC 360 (containing 120 g/L flufenacet + 120 g/L diflufenican + 120 g/L flurtamone) in southern Europe**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
12-2002  12-2002-01 GLP: yes 2011	Barley, winter Platine	France 13103 Saint Etienne du gres Europe, South	360 SC	1	0.12	0.040	25	green material whole plant without roots grain straw	121 155 192 192	0.035 0.045 <0.01 0.069
12-2002  12-2002-02 GLP: yes 2012	Barley, winter Amillis	Italy 37050 Perzacco Europe, South	360 SC	1	0.12	0.040	23	green material whole plant without roots grain straw	46 76 105 105	0.027 0.025 <0.01 <0.05
12-2002  12-2002-04 GLP: yes 2011	Wheat, winter Hystar	Portugal 2005-009 Casais da Narcisa Europe, South	360 SC	1	0.12	0.040	22	green material whole plant without roots grain straw	129 185 213 213	<0.01 <0.01 <0.01 <0.05

\*Residues for total residue flufenacet (determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet)

DALT : Days after last treatment

### Conclusion:

Three trials on winter wheat (1) and winter barley (2) were performed during the 2011/2012 growing season in southern Europe to investigate the residues of flufenacet (and flurtamone) in cereals after application of 120 g flufenacet/ha using a triple mixture also containing flurtamone and diflufenican. The plants were treated at growth stages BBCH 22-25 in autumn/winter depending on the crop development. Residues of flufenacet ranged between < 0.01 and 0.035 mg/kg in green plant material at forage stage and between < 0.01 and 0.045 mg/kg at silage stage (whole plant without root). At harvest, flufenacet derived residues in grain were < 0.01 mg/kg and ranged from < 0.05-0.069 mg/kg in straw.

The trials are considered appropriate to support the representative GAP in southern Europe with an application rate of 160 g as/ha since the deviation to the rate of the supported GAP is within the EU's tolerance criteria for comparability (-25%).

**B.7.3.1.2.7 Supplementary field trials in southern Europe (application rate flufenacet: 126 g as/ha)**

<b>Report:</b>	<b>KCA 6.3.1/04</b> , Seym, M.; Deissler, A.; 1999; M-012486-02_
Title:	Determination of the residues of FOE 5043 & Diflufenican 70 WG in/on winter barley and winter wheat in the field in France
Document No Report No	<b>M-012486-02-1</b> Study No. RA-2153/97 dated 1999-07-29
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed
GLP	Yes ; Deviations : none

**Material and methods**

Three trials on winter wheat (1) or winter barley (2) were conducted during the 1997/1998 growing season in southern France using a WG formulation containing 35% flufenacet and 35% diflufenican (WG 70). The plants were treated in late autumn (November, December) at growth stage BBCH 13 (3 leaves unfolded). The application rate for flufenacet was 126 g as/ha.

Grain and straw samples were taken at normal harvest, which was between 209 and 229 days after application.

All samples were analysed for residues of flufenacet according to the method 00346 (Seym, M.; 1995; M-018864-02), which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. The procedure involves oxidation of the residues with potassium permanganate, hydrolysis with sulfuric acid, steam distillation, liquid/liquid partitioning, derivatisation with trifluoroacetic anhydride and GC/MS determination of the thus obtained 2,2,2-trifluoro-*N*-(4-fluorophenyl)-*N*-isopropylacetamide (trifluoroacetamide). Residues are expressed as parent flufenacet. The method was evaluated with the original Annex II dossier.

The limit of quantification (LOQ) of flufenacet was 0.05 mg/kg in grain and in straw. Other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrently with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw.

Before analysis for flufenacet the samples were stored frozen up to 3.5 months (106 days) for grain and straw. The storage period is adequately covered by the storage stability data for flufenacet.

**Findings**

The accuracy of the residue determination was established by determining recoveries prior to analysis in order to validate the method and by procedural recoveries from control samples of straw and grain fortified with flufenacet. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The average recoveries and relative standard deviations (RSD) were satisfactory as shown in Table 7.3.1.2.7-1.

Residues for flufenacet were < LOQ in untreated control samples. The residues found in the wheat and barley samples from the individual trials are summarised in Table 7.3.1.2.7-2. Flufenacet derived residues in grain and straw were less than the LOQ in all trials.

**Table 7.3.1.2.7-1: Procedural recovery data for Flufenacet** (the LOQ is marked in bold).

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2153/97 70258/7 0258-97  70731/7 0731-97  GLP: yes 1997	Barley, winter	grain	total residue flufenacet	9	<b>0.05</b>	74; 77; 82; 82; 82; 82; 83; 83; 93	74	93	82	6.3
				9	overall mg/kg		74	93	82	6.3
		straw	total residue flufenacet	11	<b>0.05</b>	77; 86; 89; 90; 92; 94; 94; 94; 94; 103; 109	77	109	93	8.9
				11	overall mg/kg		77	109	93	8.9
RA-2153/97 70732/5 0732-97 GLP: yes 1997	Wheat, winter	grain	total residue flufenacet	11	<b>0.05</b>	75; 76; 76; 76; 78; 78; 80; 80; 81; 87; 88	75	88	80	5.5
				11	overall mg/kg		75	88	80	5.5
		straw	total residue flufenacet	9	<b>0.05</b>	80; 80; 82; 90; 92; 94; 94; 95; 95	80	95	89	7.4
				9	overall mg/kg		80	95	89	7.4

Fortified with flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide or a mixture thereof; determined as FOE 5043 trifluoroacetamide and calculated as flufenacet equivalent

**Table 7.3.1.2.7-2: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican WG 70 (containing 35% flufenacet + 35% diflufenican) in southern Europe**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2153/97 70258/7 0258-97 GLP: yes 1997	Barley, winter Vertige	France F-01440 Viriat Europe, South	70 WG	1	0.126	0.045	13	grain	215	<0.05
								straw	215	<0.05
RA-2153/97 70731/7 0731-97 GLP: yes 1997	Barley, winter Pastoral	France F-01190 St. Benigne Europe, South	70 WG	1	0.126	0.045	13	grain	229	<0.05
								straw	229	<0.05
RA-2153/97 70732/5 0732-97 GLP: yes 1997	Wheat, winter Soissons	France F-01190 St. Benigne Europe, South	70 WG	1	0.126	0.045	13	grain	209	<0.05
								straw	209	<0.05

\*Residues for total residue flufenacet (determined as FOE 5043 Trifluoroacetamide and calculated as flufenacet)  
 DALT : Days after last treatment

## Conclusion

Three trials on winter cereals (2 trials on barley, 1 trial on winter wheat) were conducted during the 1997-1998 growing season in southern France to investigate the residues of flufenacet in cereals after application of 126 g flufenacet/ha (and 126 g diflufenican/ha) using a mixed WG formulation of the two substances. The plants were treated in autumn (November, December), at growth stage BBCH 13 (3 leaves unfolded). At mature harvest, the residues of flufenacet were < 0.05 mg/kg in grain and in straw.

The trials are considered appropriate to support the representative GAP in southern Europe with an application rate of 160 g as/ha since the deviation to the rate of the supported GAP is within the EU's tolerance criteria for comparability (-21%).

<b>Report:</b>	<b>KCA 6.3.1/05</b> , Neigl, A.; 2000; M-033163-01_
Title:	Determination of residues of FOE 5043 on winter wheat after spray application of FOE 5043 & Diflufenican 70 WG in the field in France
Document No Report No	<b>M-033163-01-1</b> Study No. RA-2185/98 dated 2000-05-12
Guidelines:	Directive 94/414/EEC Residues in or on treated products, food and feed
GLP	Yes ; Deviations : none

## Material and methods

Two trials on winter wheat were conducted during the 1998/1999 growing season in southern France using a WG formulation containing 35% flufenacet and 35% diflufenican (WG 70). The plants were treated in

late autumn (October, December) at growth stage BBCH 13 (3 leaves unfolded). The application rate of flufenacet was 126 g as/ha.

Grain and straw samples were taken at normal harvest, which was between 206 and 266 days after application.

All samples were analysed for residues of flufenacet according to the method 00346 (Seym, M.; 1995; M-018864-02), which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group (see above).

The limit of quantification (LOQ) of flufenacet was 0.05 mg/kg in grain and in straw. Other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrently with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw.

Before analysis for flufenacet the samples were stored frozen up to 4.5 months (138 days) for grain and straw. The storage period is covered by the storage stability data for flufenacet.

### Findings

The accuracy of the residue determination was established by determining recoveries prior to analysis in order to validate the method and by procedural recoveries from control samples of straw and grain fortified with flufenacet. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The average recoveries and relative standard deviations (RSD) were satisfactory as shown in Table 7.3.1.2.7-3.

Residues for flufenacet were < LOQ in untreated control samples. The residues found in the wheat samples from the individual trials are summarised in Tables 7.3.1.2.7-4. Flufenacet derived residues in grain and straw were less than the LOQ in both trials.

**Table 7.3.1.2.7-3: Procedural recovery data for Flufenacet (the LOQ is marked in bold).**

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2185/98 R 1998 1726/0 1726-98 and R 1998 1727/9 1727-98  GLP: yes 1998	Wheat, winter	grain	total residue flufenacet	11	<b>0.05</b>	76; 80; 81; 81; 82; 82; 83; 85; 85; 86; 90	76	90	83	4.4
				11	overall mg/kg		76	90	83	4.4
		straw	total residue flufenacet	9	<b>0.05</b>	77; 78; 80; 82; 85; 86; 88; 89; 92	77	92	84	6.2
				9	overall mg/kg		77	92	84	6.2

Fortified with flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide or a mixture thereof; determined as FOE 5043 trifluoroacetamide and calculated as flufenacet equivalent

**Table 7.3.1.2.7-4: Residues of flufenacet in wheat and barley after post-emergence application of flufenacet + diflufenican WG 70 (containing 35% flufenacet + 35% diflufenican) in southern Europe**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2185/98 R 1998 1726/0 1726-98 GLP: yes 1998	Wheat, winter Sideral	France F-01380 Bage-La Ville Europe, South	70 WG	1	0.1260	0.04515	13	grain	266	<0.05
								straw	266	<0.05
RA-2185/98 R 1998 1727/9 1727-98 GLP: yes 1998	Wheat, winter Isangrain	France F-01190 Saint Benigne Europe, South	70 WG	1	0.1260	0.04515	13	grain	206	<0.05
								straw	206	<0.05

\*Residues for total residue flufenacet (determined as FOE 5043 Trifluoroacetamide and calculated as flufenacet)  
DALT : Days after last treatment

## Conclusion

Two trials on winter wheat were conducted during the 1998-1999 growing season in southern France to investigate the residues of flufenacet in cereals after application of 126 g flufenacet/ha using a mixed WG formulation with diflufenican. The plants were treated in autumn (October, December), at growth stage BBCH 13 (3 leaves unfolded). At mature harvest, the residues of flufenacet were < 0.05 mg/kg in grain and in straw.

The trials are considered appropriate to support the representative GAP in southern Europe with an application rate of 160 g as/ha since the deviation to the rate of the supported GAP is within the EU's tolerance criteria for comparability (-21%).

## Summary conclusion for the use of flufenacet with use patterns involving 120 g flufenacet/ha in northern Europe and 160 g as/ha in southern Europe (B.7.3.1.2.4 – B.7.3.1.2.7)

The data set evaluated for Annex I inclusion was considered suitable to support a use pattern at 240 g as/ha in northern Europe with application in autumn. In the evaluated trials winter cereals were treated between November and March at growth stages ranging from BBCH 11 to 25. In all trials, residues have shown to be less than the LOQ for grain (< 0.05 mg/kg) and straw (< 0.1 mg/kg).

With this dossier 6 supplementary residue trials are reported where flufenacet has been applied in mixture with diflufenican with WG and SC formulations at growth stages ranging between BBCH 13 and 25. Thus, a large data set of 23 trials is available to support the representative use at 240 g as/ha.

This data set is considered appropriate to support the critical GAP involving 240 g as/ha for the northern climatic zone. Since the second representative use involving an application rate of 120 g as/ha can be considered to be less critical the data set supporting the high rate can be considered suitable to also support the lower application rate by establishing the risk envelope.

However, 8 supplementary trials are reported with the present dossier with mixture products which support a use pattern for the representative use at 120 g as/ha at growth stages up to BBCH 22 (actual BBCH 22-25). Since a slightly later growth stage is targeted the growth stage was given priority in the design of the residue trials, because the application may not always be possible in autumn. Thus the residue trials cover scenarios with treatments between November and April. The findings demonstrate that residue levels range between the lowered LOQ ( $< 0.01$  mg/kg) and 0.022 mg/kg (median  $< 0.01$  mg/kg) in grain and remain below the lower LOQ ( $< 0.05$  mg/kg) in straw. It may be concluded that a possibly later application does not result in higher residues at a rate of maximum 120 g as/ha.

For the southern European climatic zone a data set of 9 trials is reported involving an application rate of 240 g as/ha and application at growth stage BBCH 13 and BBCH 21 in one trial. The trials were all performed using the representative formulation 'Flufenacet + Diflufenican SC 600'. In the southern zone cereals are typically sown continuously during autumn and winter. Thus, in order to reflect this agricultural practice applications were made early post-emergence between December and February. Residue levels in grain ranged between  $< 0.01$  and 0.05 mg/kg (median  $< 0.01$  mg/kg) and  $< 0.05$  and 0.11 mg/kg (median 0.06 mg/kg) in straw.

This GAP can be considered as the critical GAP for the southern region. This data set is considered appropriate to cover the less critical GAP of the second representative use for the southern region at a rate of 160 g as/ha by establishing the risk envelope. However, 12 supplementary trials are submitted using mixture products. The application rates for these trials were 21-25% less relative to the target rate (160 g as/ha), however within the 25% range of the tolerance criteria for comparability. Treatments were made between October and March at growth stages ranging from BBCH 13 to 30. Residue levels ranged between less than the LOQ ( $< 0.01$  or  $< 0.05$  mg/kg) to 0.035 mg/kg in grain and  $< 0.05$  to 0.069 mg/kg in straw. The supplementary trials broaden the database and confirm the representative use at 240 g as/ha as the critical GAP.

### B.7.4. FEEDING STUDIES

#### Studies evaluated during Annex I submission and reconsidered for renewal of active substance approval.

During the EU evaluation process the dietary burden for livestock was assessed based on uses in cereals, corn, sunflower and soybean as relevant feeding items. Since i) no residues above the LOQ (0.05 mg/kg in green material of plants (at forage stage), cereal grain, sunflower and soybean seed, maize kernel and 0.1 mg/kg in straw) were determined and ii) the data from metabolism studies do not indicate a possible transfer from residues in feeding items to food of animal origin, it was concluded in the Monograph that livestock feeding studies are not required.

However, a cow feeding study conducted for the US was submitted and has been evaluated. In this study, cows were administered highly exaggerated doses of FOE5043-oxalate which constitutes the main plant metabolite. The results show that even at an exaggerated dose of 7.8 ppm (14N; 1N dose; 0.555 ppm; see Table 7.4-2) no flufenacet derived residues can be expected in tissues or products of animals which have been fed flufenacet treated crops.

<b>Report:</b>	██████████ (1995): FOE oxalate - A 29-day dairy cattle feeding study; Bayer Corp., unpublished report no.: 106945 of September 27, 1995
<b>Guidelines:</b>	EPA Pesticide Assessment Guidelines, 171-4(j); Magnitude of the Residue -Meat/Milk
<b>GLP:</b>	Yes

In the Report of ECCO 73, Annex 2, Complete List of Endpoints, it is concluded that no residues can be expected in animal tissues or products and, thus, it was proposed to delete all MRLs for products of animal origin.

#### Evaluation in the EFSA Reasoned Opinion on the review of the existing maximum residue levels (MRLs) for flufenacet according to Art 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689

Based on the uses reported by the RMS, significant intakes were calculated for ruminants, poultry and pigs. EFSA calculated the dietary burden based on all authorized uses for crops that might be fed to livestock (potatoes, sunflower seed, soya bean, barley, maize, rye, wheat) and the corresponding by-products which may be used as feeding items (cereal bran, oilseed meals). In the EFSA Reasoned Opinion, the median and maximum dietary burdens were therefore calculated for different groups of livestock using the agreed European methodology (EC, 1996). The input values for all relevant commodities have been selected according to the recommendations of JMPR (FAO, 2009) and are summarized in Table 7.4-1 (corresponds to Table 3-4 of the Reasoned Opinion). For cereal bran and sunflower seed meal default processing factors of 8 and 2, respectively, have been included in the calculation in order to consider potential concentration of residues in these commodities. The default processing factor for soya bean has not been applied as processing studies submitted with the Annex II dossier show that residues of flufenacet are below the LOQ in both the RACs and the processed products and no concentration of flufenacet is observed.

**Table 7.4-1: Input values for the dietary burden calculation**

Commodity	Median dietary burden		Maximum dietary burden	
	Input value (mg/kg)	Comment	Input value (mg/kg)	Comment
Cereal grain (small)	0.05	Median residue	0.05	Median residue
Maize grain	0.05	Median residue	0.05	Median residue
Cereal bran	0.4	Median residue × 8	0.4	Median residue × 8
Cereal straw	0.1	Median residue	0.11	Highest residue
Potatoes	0.05	Median residue	0.11	Highest residue
Sunflower seed	0.05	Median residue	0.05	Median residue
Sunflower seed meal	0.1	Median residue × 2	0.1	Median residue × 2
Soya bean	0.05	Median residue	0.05	Median residue
Soya bean meal	0.05	Median residue	0.05	Median residue

The results of the calculations are reported in Table 7.4-2 (corresponds to Table 3-5 of the Reasoned Opinion). The calculated dietary burdens for all groups of livestock were found to exceed the trigger value of 0.1 mg/kg DM.

**Table 7.4-2: Results of the dietary burden calculation**

	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Dairy ruminants	0.0090	0.0135	Potatoes	0.3704	Y
Meat ruminants	0.0134	0.0238	Potatoes	0.5555	Y
Poultry	0.0092	0.0143	Wheat bran	0.2257	Y
Pigs	0.0125	0.0221	Potatoes	0.5531	Y

Evaluation of the magnitude of residues in livestock (EFSA Reasoned Opinion, 2012):

*“On the basis of the animal metabolism studies it is concluded that, after exposure to the maximum dietary burden (about 200 times lower than the dose level in the metabolism studies, [5 mg/kg bw/d]), residue levels in livestock commodities are expected to remain below the enforcement LOQ of 0.01 mg/kg in milk, 0.02 mg/kg in liver and 0.05 mg/kg in fat, eggs, kidney and muscle. Hence, no livestock feeding study is needed; MRLs and risk assessment values for the relevant commodities in ruminants, pigs and poultry can be established at the LOQ level.” (p.29/30).*

The EFSA Reasoned Opinion confirms the conclusion drawn in the EU review process relative to the evaluation of the dairy cattle feeding study conducted in accordance with the US EPA guidance: *“The results of the study show that no detectable residues of flufenacet oxalate are to be expected in products of animal origin which have been fed crops treated with flufenacet according to the GAPs (...).*

The lowest dose rate in the US feeding study amounted to 7.8 mg/kg in feed corresponding to 14 times the maximum dietary burden for meat ruminants (0.555 mg/kg DM) and 21 times the dietary burden for dairy ruminants (0.370 mg/kg DM) as shown in the calculation above.

In animal tissues, FOE oxalate residues were only detected at the highly exaggerated treatment rates.

At the lowest dosing rate rate (14N), only the kidney just barely showed a measurable residue (up to 0.057 mg/kg). Therefore, no detectable residues of flufenacet are to be expected in meat from cattle, which are fed at the 1× rate.

In milk no residues above the LOQ (= 0.01 mg/kg) was found even in samples obtained from cows fed at the highest dose rate (148× rate corresponding to 82 mg/kg feed).

It has to be noted that the applicant intends to limit the flufenacet uses to cereals, potatoes and maize, thus resulting in a slightly lower dietary burden for dairy ruminants, poultry and pigs but without any impact on the dietary burden for meat ruminants (see Table 7.4-3).

**Table 7.4-3: Results of the dietary burden calculation (based on supported crops cereals, maize, potato)**

	Median dietary burden (mg/kg bw/d)	Maximum dietary burden (mg/kg bw/d)	Highest contributing commodity	Max dietary burden (mg/kg DM)	Trigger exceeded (Y/N)
Dairy ruminants	0.0078	0.0122	Potatoes	0.3355	Y
Meat ruminants	0.0134	0.0238	Potatoes	0.5555	Y
Poultry	0.0084	0.0135	Potatoes	0.2141	Y
Pigs	0.0116	0.0212	Potatoes	0.5299	Y

#### Calculation of the dietary burden according to the OECD guidance document on residues in livestock No 73 (4 September 2013)

The new EU data requirement as published with Regulation (EC) 283/2013 for active substances state the need for feeding studies where intake is above 0.004 mg/kg bw/d. However, the circumstances in which feeding studies are required also have to take into consideration where metabolism studies indicate that

residues at levels of above 0.01 mg/kg may not occur in edible animal tissue, milk, eggs or fish, taking into account the residue levels in potential feeding items, obtained at the  $1 \times$  dose rate, calculated on the dry weight basis.

Table 7.4-4 compiles the input data for the dietary burden calculation relevant to the representative uses on small grain cereals. Following the recommendation in the EFSA document ‘Estimation of animal intakes and HR, STMR and MRL calculations for products of animal origin’ (September 2015, issued on the DG SANTE website) uses on cereals are – by default – understood as ‘uses on cereals for grain production’ and therefore, only residues in grains and straw from cereals shall be considered for the animal dietary burden calculation below.

Table 7.4-5 provides the results of the dietary burden calculation for Europe according to the OECD guidance document on residues in livestock (ENV/JM/MONO(2013)8, 04-Sep-2013) and the feeding tables provided on the DG SANTE website (version 2015) and by using the RWCF approach (Reasonable Worst Case Feed).

**Table 7.4-4: Input data for dietary burden calculation according to OECD guidance document**

Category	Crop	Commodity	IFN Code	Classification	Residue input	Dry matter (%)	Residue level on fresh / total weight basis (mg/kg) EU
Forages / Fodders	Barley	straw	1-00-498	R	HR	89	0.110
Forages / Fodders	Oat	straw	1-03-283	R	HR	90	0.110
Forages / Fodders	Rye	straw	1-04-007	R	HR	88	0.110
Forages / Fodders	Triticale	straw	NA	R	HR	90	0.110
Forages / Fodders	Wheat	straw	1-05-175	R	HR	88	0.110
Cereal grains / Crop Seeds	Barley	grain	4-00-549	CC	STMR	88	0.050
Cereal grains / Crop Seeds	Oat	grain	4-03-309	CC	STMR	89	0.050
Cereal grains / Crop Seeds	Rye	grain	4-04-047	CC	STMR	88	0.050
Cereal grains / Crop Seeds	Triticale	grain	4-20-362	CC	STMR	89	0.050
Cereal grains / Crop Seeds	Wheat	grain	4-05-211	CC	STMR	89	0.050
By-products	Wheat gluten	meal	5-05-221	CC	STMR	40	0.050 <sup>a</sup>
By-products	Wheat	milled by products	4-06-749	CC	STMR	88	0.22 <sup>a</sup>

<sup>a</sup> for derivation of processing factors, please refer to B 7.5.3.

For wheat gluten meal a processing factor < 1 was derived. For the calculation of the dietary burden a processing factor of 1 has been used.

For milled by-products from wheat a worst case processing factor (mean) of 4.4 derived from bran and shorts has been used.

Table 7.4-5: Results of the dietary burden calculation for flufenacet in Europe (OECD guidance, 2013, spreadsheet from DG SANTE website; 2015)

Maximum Intake (mg/kg bw/d)	Cattle						Sheep					
	Beef			Dairy			Ram/Ewe			Lamb		
	500 kg 12 kg			650 kg 25 kg			75 kg 2.5 kg			40 kg 1.7 kg		
	0.003	mg/kg bw/d	%	0.005	mg/kg bw/d	%	0.006	mg/kg bw/d	%	0.008	mg/kg bw/d	%
Contributor 1	Wheat	milled bypds	30	Wheat	milled bypds	30	Wheat	milled bypds	40	Wheat	milled bypds	50
Contributor 2	Barley	straw	30	Barley	straw	30	Barley	straw	60	Barley	straw	50
Contributor 3	Barley	grain	40	Barley	grain	40			0			0
Contributor 4												
Median intake	0.0028	mg/kg bw/d		0.0045	mg/kg bw/d		0.0047	mg/kg bw/d		0.0067	mg/kg bw/d	

Maximum Intake (mg/kg bw/d)	Swine					
	Breeding			Finishing		
	260 kg 6 kg			100 kg 3 kg		
	0.004	mg/kg bw/d	%	0.005	mg/kg bw/d	%
Contributor 1	Wheat	milled bypds	50	Wheat	milled bypds	50
Contributor 2	Barley	grain	50	Barley	grain	50
Contributor 3						
Contributor 4						
Median intake	0.004	mg/kg bw/d		0.005	mg/kg bw/d	

Intakes &gt;0.004 mg/kg bw/d are highlighted

Maximum Intake (mg/kg bw/d)	Poultry								
	Broiler			Layer			Turkey		
	1.7 kg 0.12 kg			1.9 kg 0.13 kg			7 kg 0.5 kg		
	0.006	mg/kg bw/d	%	0.007	mg/kg bw/d	%	0.006	mg/kg bw/d	%
Contributor 1	Wheat	milled bypds	20	Wheat	milled bypds	20	Wheat	milled bypds	20
Contributor 2	Barley	grain	70	Wheat	straw	10	Rye	grain	60
Contributor 3				Barley	grain	70			
Contributor 4									
Median intake	0.006	mg/kg bw		0.007	mg/kg bw		0.006	mg/kg bw	

Intakes expressed on the dry mater basis (mg/kg DM)						
mg/kg DM	Cattle		Sheep		Swine	
	Beef	Dairy	Ram/Ewe	Lamb	Breeding	Finishing
Maximum	0.13	0.13	0.17	0.19	0.15	0.15
Median	0.12	0.12	0.14	0.16	0.15	0.15
	Poultry			Intake >0.1 mg/kg DM in red characters		
	Broiler	Layer	Turkey			
Maximum	0.09	0.10	0.08			
Median	0.09	0.10	0.08			

## Conclusion

As outlined above, during the EU peer review process and recently concluded in the EFSA Reasoned Opinion on existing MRLs (2012) the transfer of flufenacet derived residues into animal tissues, milk and eggs is very low and no residues above the respective LOQs can be expected based on the evaluated GAPs. The representative uses on cereals supported in the present dossier are shown not to produce higher residues than those previously evaluated.

The conclusion was drawn based on the available metabolism data obtained after a dose 200 times the maximum dietary burden for ruminants and 350 times the maximum dietary burden for poultry and using 3 different labels (fluorophenyl-U-<sup>14</sup>C label, thiadiazole-2-<sup>14</sup>C label and fluorophenyl-U-<sup>14</sup>C flufenacet oxalate (main plant metabolite)). The metabolism studies with fluorophenyl-UL-<sup>14</sup>C flufenacet oxalate - showing by far the lowest transfer into animal tissues, milk and eggs - are considered to provide the most relevant information because the parent compound is rapidly metabolized and no parent is found in plant commodities. Taking into account the findings from the ruminant feeding study, the lowest dose of flufenacet oxalate fed to cows amounted to 0.22 mg/kg bw/d corresponding to 9 times the maximum dietary burden calculated for meat cattle (0.0238 mg/kg bw/day; cf. Table 7.4-3).

In addition to the European methodology applied by EFSA (2012), the dietary burden was calculated according to the OECD guidance document (2013) taking into account the most recent feeding tables and using the calculation spreadsheet issued on the DG SANTE website (version 2015). Based on the feeding items originating from small grain cereals (including by-products) the dietary burden calculated for livestock was up to a maximum of 0.008 mg/kg bw/d (sheep - lamb).

The conclusions drawn for Annex I inclusion and in the EFSA Reasoned Opinion on existing MRLs are considered to be still valid and no further data are considered necessary for this submission.

### B.7.4.1. Poultry

No supplementary study has been generated.

### B.7.4.2. Ruminants

No supplementary study has been generated.

### B.7.4.3. Pigs

The metabolic pathway of flufenacet was similar in rats, poultry (laying hens), and ruminants (goat). Therefore, it can be expected that the metabolism in other farm animals does not differ, and thus for the active substance studies in pigs are not required.

### B.7.4.4. Fish

The nature of the residue in fish was addressed in chapter 7.2.4 above based on an available bio-concentration study with bluegill sunfish also reporting metabolism data in fish.

No final test guideline or feeding tables are currently available which detail how the dietary burden has to be calculated and which provide an agreed test methodology. Therefore it is the opinion of the applicant that it is not appropriate to address this issue until such guidance is available. Therefore the risk assessment should be conducted in accordance with the current published guidelines.

This opinion is in agreement with a publication of European Commission Health & Consumer Protection Directorate-General published as SANCO/10181/2013-rev. 2 of 2-May-2013 on “Guidance Document for Applicants on Preparing Dossiers for the Approval of a Chemical New Active Substance and For the Renewal of Approval of a Chemical Active Substance According to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013”.

This SANCO document notes in Section 4. “Documents to be included in a submission” under the Subsection “Special cases”:

*“In some cases, agreed test methods of guidance documents are not yet available for particular data requirements. In these cases, waving of these particular data requirement points is considered acceptable as long as no test methods or guidance documents are published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C95/02.”*

#### B.7.4.5. Thiadone metabolites intake

Dietary burden calculations were performed for thiadone-*N*-glucoside and TFA according to the OECD calculation sheet (2016). Input values, maximum dietary burdens are presented in Tables 7.4.5-1 to 7.4.5-6.

#### Thiadone-*N*-glucoside (THNG, M25)

**Table 7.4.5-1 Input values for Thiadone-*N*-glucoside (THNG, M25)**

Commodity	Residue level (mg equ/kg) scaled to 240 g as/ha	Extrapolation from
Forages except cereals	0.043	CRC, Turnip leaves 1 <sup>st</sup> rot
Forages (hay) except cereals	0.038	CRC, Cereal hay 1 <sup>st</sup> rot
Forages (stover & straw) except cereals	0.053	CRC, Cereal straw 1 <sup>st</sup> rot.
Cereal straw	1.0	Wheat metabolism (primary)
Cereal forage	1.11	Wheat metabolism (primary)
Root & tubers	0.014	CRC, Turnip body 1 <sup>st</sup> rot.
Cereal grains	0.003	Wheat metabolism (primary)
Other grain & seeds	--	no residues in rot crops

The following maximum dietary burden burdens are calculated (if feed items would originate from primary treated crops and after a crop failure situation from rotational crops).

**Table 7.4.5-2 Maximum dietary burdens for THNG (M25)**

Livestock species	Dietary burden (mg equ/kg bw/d)	Dietary burden (mg THNG/kg bw/d)	Overdose factor in goat metabolism study (0.43 mg THNG/kg bw/d)
Beef	0.028	0.021	20.5
Dairy	0.044	0.034	12.6
Swine	0.002	0.0015	287

Molweight THNG 278.28

Molweight flufenacet: 363.33

**Table 7.4.5-3: Results of the dietary burden calculation for Thiadone-*N*-glucoside metabolite in Europe (OECD guidance, 2013, spreadsheet from DG SANTE website; 2016)**

Animal burden calculation							Thiadone- <i>N</i> -glucoside metabolite from flufenacet				
According to: "OECD Guidance Document, Series on testing and assessment No 64 and Series on pesticides No 32" and "OECD Guidance Document on Residues in livestock, Series on Pesticides No 73"											
Maximum Intake (mg/kg bw/d)	Cattle						Sheep				
	Beef			Dairy			Ram/Ewe			Lamb	
	500 kg 12 kg			650 kg 25 kg			75 kg 2,5 kg			40 kg 1,7 kg	
	0,028	mg/kg bw/d	%	0,044	mg/kg bw/d	%	0,063	mg/kg bw/d	%	0,080	mg/kg bw/d
Contributor 1	Barley	forage	30	Barley	forage	30	Barley	forage	50	Barley	forage
Contributor 2	Swede	roots	40	Swede	roots	20	Swede	roots	30	Swede	roots
Contributor 3	Barley	grain	30	Barley	grain	40	Barley	grain	20	Barley	grain
Contributor 4											
Median intake	0,0280	mg/kg bw/d		0,0438	mg/kg bw/d		0,0631	mg/kg bw/d		0,0804	mg/kg bw/d

Maximum Intake (mg/kg bw/d)	Swine					
	Breeding			Finishing		
	260 kg 6 kg			100 kg 3 kg		
	0,002	mg/kg bw/d	%	0,002	mg/kg bw/d	%
Contributor 1	Grass	forage (fresh)	20	Lespedeza	forage	10
Contributor 2	Swede	roots	40	Swede	roots	40
Contributor 3	Barley	grain	40	Barley	grain	50
Contributor 4						
Median intake	0,002	mg/kg bw/d		0,002	mg/kg bw/d	

Maximum Intake	Poultry				
	Broiler		Layer		Turkey
	1,7 kg 0,12 kg		1,9 kg 0,13 kg		7 kg 0,5 kg

Intakes >0.004 mg/kg bw/d are highlighted

**Flufenacet**

**Volume 3 – B.7 (AS)**

(mg/kg bw/d)	0,001	mg/kg bw/d	%	0,009	mg/kg bw/d	%	0,001	mg/kg bw/d	%
Contributor 1	Swede	roots	10	Wheat	straw	10	Potato	culls	20
Contributor 2	Barley	grain	70	Swede	roots	10	Rye	grain	60
Contributor 3				Barley	grain	80			
Contributor 4									
<b>Median intake</b>	0,001	mg/kg bw		0,009	mg/kg bw		0,001	mg/kg bw	

Intakes expressed on the dry mater basis (mg/kg DM)						
mg/kg DM	Cattle		Sheep		Swine	
	Beef	Dairy	Ram/Ewe	Lamb	Breeding	Finishing
<b>Maximum</b>	1,17	1,14	1,9	1,89	0,09	0,08
<b>Median</b>	1,17	1,14	1,89	1,89	0,09	0,08
	<b>Poultry</b>			<b>Intake &gt;0.1 mg/kg DM</b> <b>in red characters</b>		
	Broiler	Layer	Turkey			
<b>Maximum</b>	0,02	0,13	0,02			
<b>Median</b>	0,02	0,13	0,02			

**TFA****Table 7.4.5-4 Input values for TFA**

Commodity	Residue level (mg TFA-Na/kg) scaled to 240 g as/ha	Extrapolation from
Forages	0.640	CRC, Turnip leaves 1.rot
Cereal straw	0.916	CRC, Wheat 2 <sup>nd</sup> rot
Root & tubers	0.050	CRC, Turnip body 1 <sup>st</sup> rot.
Grains & seeds	0.759	CRC, Wheat 2 <sup>nd</sup> rot

**Table 7.4.5-5 Maximum dietary burdens for TFA**

Livestock species	Dietary burden (mg TFA-Na/kg bw/d)	Dietary burden (mg TFA/kg bw/d)
Beef	0.035	0.29
Dairy	0.073	0.061
Swine	0.031	0.026
Poultry (layer)	0.071	0.059

Table 7.4.5-6: Results of the dietary burden calculation for TFA (OECD guidance, 2013, spreadsheet from DG SANTE website; 2016)

Animal burden calculation

TFA-Na

According to:

"OECD Guidance Document, Series on testing and assessment No 64 and Series on pesticides No 32" and "OECD Guidance Document on Residues in livestock, Series on Pesticides No 73"

Maximum Intake  (mg/kg bw/d)	Cattle						Sheep					
	Beef			Dairy			Ram/Ewe			Lamb		
	500 kg 12 kg			650 kg 25 kg			75 kg 2,5 kg			40 kg 1,7 kg		
	0,035	mg/kg bw/d	%	0,073	mg/kg bw/d	%	0,083	mg/kg bw/d	%	0,073	mg/kg bw/d	%
Contributor 1	Corn, field	forage/silage	80	Grass	forage (fresh)	60	Grass	forage (fresh)	95	Grass	forage (fresh)	50
Contributor 2	Sorghum	grain	20	Sorghum	grain	40	Sorghum	grain	5	Barley	grain	50
Contributor 3			0			0			0			0
Contributor 4												
Median intake	0,0452 mg/kg bw/d			0,0727 mg/kg bw/d			0,0825 mg/kg bw/d			0,0727 mg/kg bw/d		

Maximum Intake  (mg/kg bw/d)	Swine					
	Breeding			Finishing		
	260 kg 6 kg			100 kg 3 kg		
	0,031	mg/kg bw/d	%	0,031	mg/kg bw/d	%
Contributor 1	Beet, mangel	fodder	15	Lespedeza	forage	10
Contributor 2	Barley	grain	80	Barley	grain	80
Contributor 3	Swede	roots	5	Swede	roots	10
Contributor 4						
Median intake	0,031 mg/kg bw/d			0,031 mg/kg bw/d		

Intakes >0.004 mg/kg bw/d are highlighted

Maximum Intake	Poultry					
	Broiler		Layer		Turkey	
	1,7 kg		1,9 kg		7 kg	
	0,12 kg		0,13 kg		0,5 kg	

Intakes &gt;0.004 mg/kg bw/d are highlighted

**Flufenacet**
**Volume 3 – B.7 (AS)**

(mg/kg bw/d)	<b>0,047</b>	mg/kg bw/d	%	<b>0,071</b>	mg/kg bw/d	%	<b>0,041</b>	mg/kg bw/d	%
Contributor 1	Sorghum	grain	70	Pea	vines	10	Rye	grain	60
Contributor 2	Swede	roots	10	Barley	grain	90	Potato	culls	20
Contributor 3						0			
Contributor 4									
<b>Median intake</b>	<b>0,047</b>	mg/kg bw		<b>0,071</b>	mg/kg bw		<b>0,041</b>	mg/kg bw	

Intakes expressed on the dry mater basis (mg/kg DM)						
mg/kg DM	Cattle		Sheep		Swine	
	Beef	Dairy	Ram/Ewe	Lamb	Breeding	Finishing
<b>Maximum</b>	<b>1,46</b>	<b>1,89</b>	<b>2,5</b>	<b>1,71</b>	<b>1,36</b>	<b>1,03</b>
<b>Median</b>	1,88	1,89	2,48	1,71	1,36	1,03
	<b>Poultry</b>			<b>Intake &gt;0.1 mg/kg DM</b> <b>in red characters</b>		
	Broiler	Layer	Turkey			
<b>Maximum</b>	<b>0,67</b>	<b>1,03</b>	<b>0,57</b>			
<b>Median</b>	0,67	1,03	0,57			

Dietary burdens calculated according to OECD guidance No 73 resulted in lower levels compared to the calculations according to PROFile (former EU methodology) presented in the position paper M-476535-01-1.

## **B.7.5. EFFECTS OF PROCESSING**

### **B.7.5.1. Nature of the residue**

The effect of processing on the nature of flufenacet was investigated in the framework of the peer review. Studies on the hydrolytic degradation of flufenacet at pH 5, 7 and 9 and incubated for 30 days in the dark at 25°C (relevant to environmental conditions) showed that the parent compound is not significantly affected by this process. The residue definition in plants consists of parent flufenacet and all its derivatives and metabolites which comprise the *N*-fluorophenyl-*N*-isopropyl functional group. These residues are determined by means of the common moiety method covering all the metabolites derived from the fluorophenyl acetamide moiety. Therefore, as the residue definition in plant commodities is based on a common moiety, it seems unlikely that new metabolites not covered by the common moiety method would be generated.

All residues containing the *N*-fluorophenyl-*N*-isopropyl group in the RACs as well as each potential breakdown product containing this moiety resulting from processing of these RACs are captured by the residue analytical methods for determination of flufenacet residues. By application of these residue methods all *N*-fluorophenyl-*N*-isopropyl containing residues are hydrolysed to the analytical target 4-fluoro-*N*-isopropylaniline that is quantified by GC-MS after derivatization with TFAA or directly by HPLC-MS/MS determination. Therefore, a study on the nature of processed residues (high temperature hydrolysis according to OECD 507) resulting from use of flufenacet in crops does not provide any new information and can thus be omitted.

### **B.7.5.2. Distribution of the residue in peel and pulp**

The distribution of the residue in peel and pulp is not relevant for the small grain cereals.

### **B.7.5.3. Magnitude of residues in processed commodities**

#### **B.7.5.3.1 Studies evaluated during Annex I submission and reconsidered for renewal of active substance approval**

#### References

<b>Report:</b>	Grace, T.J. (1995a): FOE 5043 60DF - Magnitude of the residue in corn processed products; Bayer Corp. unpublished report no.: 106659 of August 2, 1995
<b>Guidelines:</b>	EPA Pesticide Assessment Guidelines, 171-4(1); Magnitude of the Residue - Processed Food/Feed
<b>GLP:</b>	Yes

<b>Report:</b>	Grace, T.J. (1995b): FOE 5043 60DF - Magnitude of the residue in soybean processed products; Bayer Corp, unpublished report no.: 106668 of August 2, 1995
<b>Guidelines:</b>	EPA Pesticide Assessment Guidelines, 171-4(1); Magnitude of the Residue - Processed Food/Feed
<b>GLP:</b>	Yes

Based on European residue data, processing studies were not considered necessary since residue levels for all edible commodities were less than the LOQ of 0.05 mg/kg and thus below the threshold of 0.1 mg/kg. However, processing studies on soybean and maize available from the US were submitted and evaluated. The active substance was applied at higher rates than in Europe (8N rate). Although residues in the raw

agricultural commodities were still below the validated LOQ of 0.05 g/kg in maize, it could be shown that no concentration of residues in any of the tested commodities occurs. The tested procedures included wet and dry milling (tested commodities starch, crude oil and refined-bleached-deodorized oil for wet milling and germs, grits, meal, flour, crude oil and refined-bleached-deodorized oil for dry milling. In soybean at the 8N rate, residues were obtained in seed. It was demonstrated that no concentration occurs in the investigated commodities meal, hulls, crude oil and refined bleached deodorized oil (see also Monograph B.6.7.2).

### B.7.5.3.2 Supplementary studies

#### WHEAT

<b>Report:</b>	<b>KCA 6.5.3/04, Noss, G.; et al.; 2013; M-457286-01</b>
<b>Title:</b>	Determination of the residues of flufenacet in/on wheat and the processed fractions (white flour, white flour bran, white bread, whole meal, whole meal bread, middlings, shorts, gluten, gluten feed meal, starch) after spraying of Flufenacet WG 60 in the United Kingdom and the Netherlands
<b>Document No</b> <b>Report No</b>	<b>M-457286-01-1</b> 11-3401 dated 2013-06-20
<b>Guidelines:</b>	<ul style="list-style-type: none"> <li>• EU-Ref: Council Directive 91/414/EEC of July 15, 1991</li> <li>• EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)</li> <li>• EC guidance working document 7035/VI/95 rev.5 (1997-07-22)</li> <li>• OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities, 508 (2008-10-03),</li> <li>• US EPA OCSPP Guideline No. 860.1520</li> </ul>
<b>GLP</b>	Yes ; Deviations : none

#### Materials and methods

Two studies were performed in 2011 on wheat in the Netherlands and the United Kingdom in order to collect sample material for processing studies. The samples of wheat (grain) to be processed were obtained after one post emergence spray application (BBCH 25) at exaggerated rate (2N = 0.48 kg as/ha) with Flufenacet WG 60, an WG formulation containing 60 % flufenacet. The higher rate was used in order to obtain appropriate residue levels in the raw agricultural commodity for derivation of transfer factors. Wheat grain samples to be processed were sampled 120-135 days after treatment, at growth stage BBCH 89.

The processing of the wheat samples into the processed fractions bran, gluten, gluten feed meal, middlings, shorts, starch A and B, wheat germ, white bread, white flour, whole meal and wholemeal bread was performed in a specialized pilot plant to simulate industrial procedures at a laboratory scale.

Residues of the raw agricultural commodity and the processed fractions were analysed using method 01100/M001 (Stuke, S., Bauer, J.; Ruhl, S.; 2012; M-433720-01) with an LOQ of 0.01 mg/kg which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

#### Processing Procedures

##### Drying / Cleaning / Conditioning of the Grain

Frozen field samples for processing were defrosted and cleaned. The grain samples were conditioned until an optimum moisture content between 14.6 to 16.6 % was reached.

##### Milling of White Flour (Type 550) and Baking of White Bread

In a closed system with different pairs of smooth rollers and sifter passages the grain was milled to straight flour, bran and middlings. Samples of bran and middlings were collected.

In a further processing step the low grade meal (toppings) were separated from the bran and middlings using a centrifuge/scouring machine. This process resulted in shorts and low grade meal. A sample of shorts was collected.

After determination of the mineral content of straight flour and low grade meal, both fractions were mixed (if necessary) to the final product white flour type 550 until a mineral content of 510-630 g/100 kg flour was reached. A sample of white flour type 550 was taken. The white flour was used to prepare white bread.

#### Milling of Whole Meal and Baking of Whole-Meal Bread

For the preparation of whole-meal and whole-meal bread the same milling procedure as used for the production of flour type 550 was used. After milling the coarse bran and middlings were cracked with an impact mill to smaller pieces. All milling products of the process were mixed homogeneously in a special flour mixer. A sample of the whole meal was collected. The whole meal flour was used to prepare whole meal bread.

#### Production of Wheat Germ

First the grain was broken to bruised grain in a special mill. The fraction 400-1000 µm, a mixture of bran, middlings and germs was put in a special separator. Due to the different specific weights of the bran, middlings and germs, the middlings/germ mixture was separated from most parts of the bran.

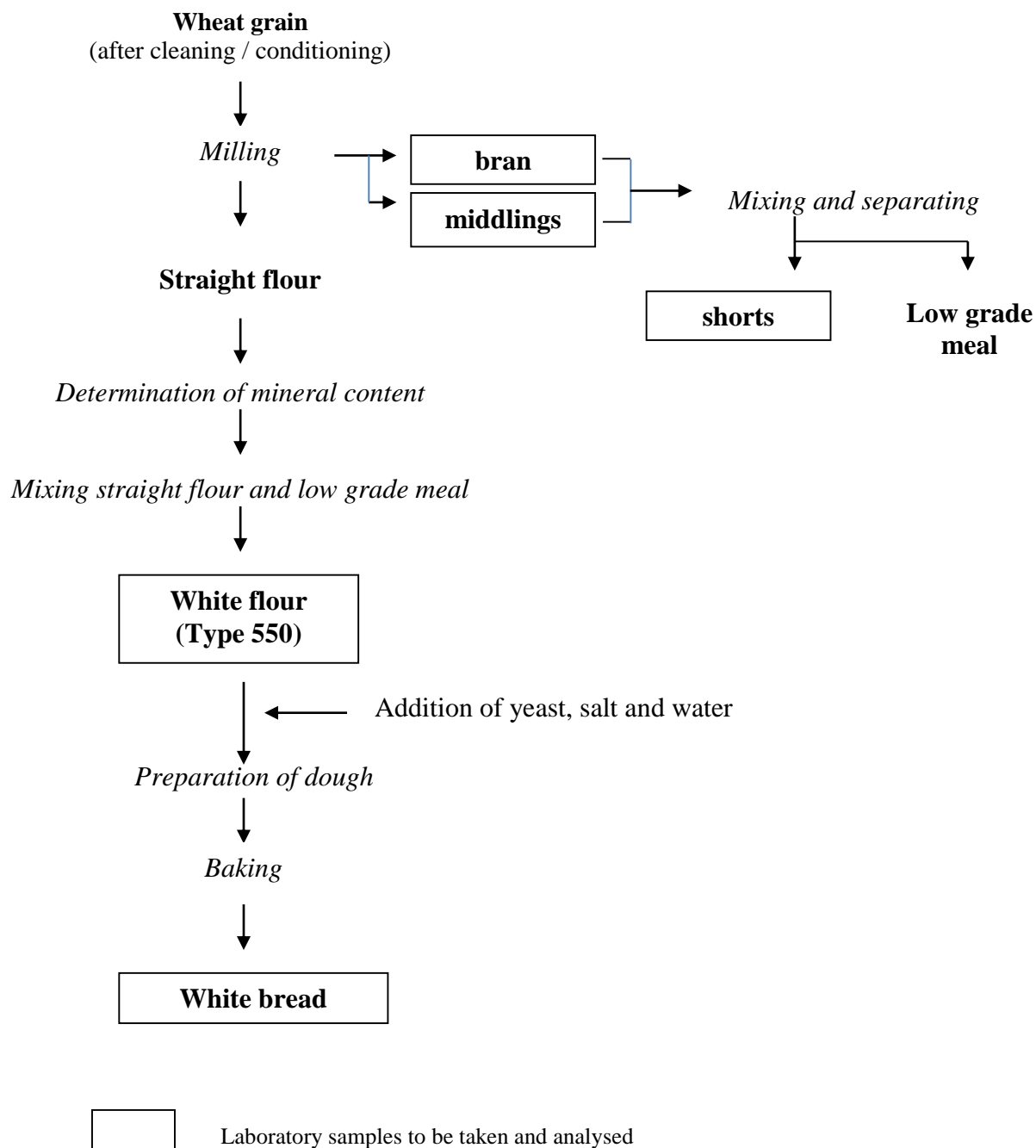
Subsequently, the middlings/germ mixture was milled to flour and small wheat germ discs incl. parts of bran in a mill with a pair of smooth rollers. The wheat germ with parts of bran was then sieved to separate the various fractions (germs with small parts of bran (germ fraction) and bran). A sample of wheat germ was taken.

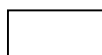
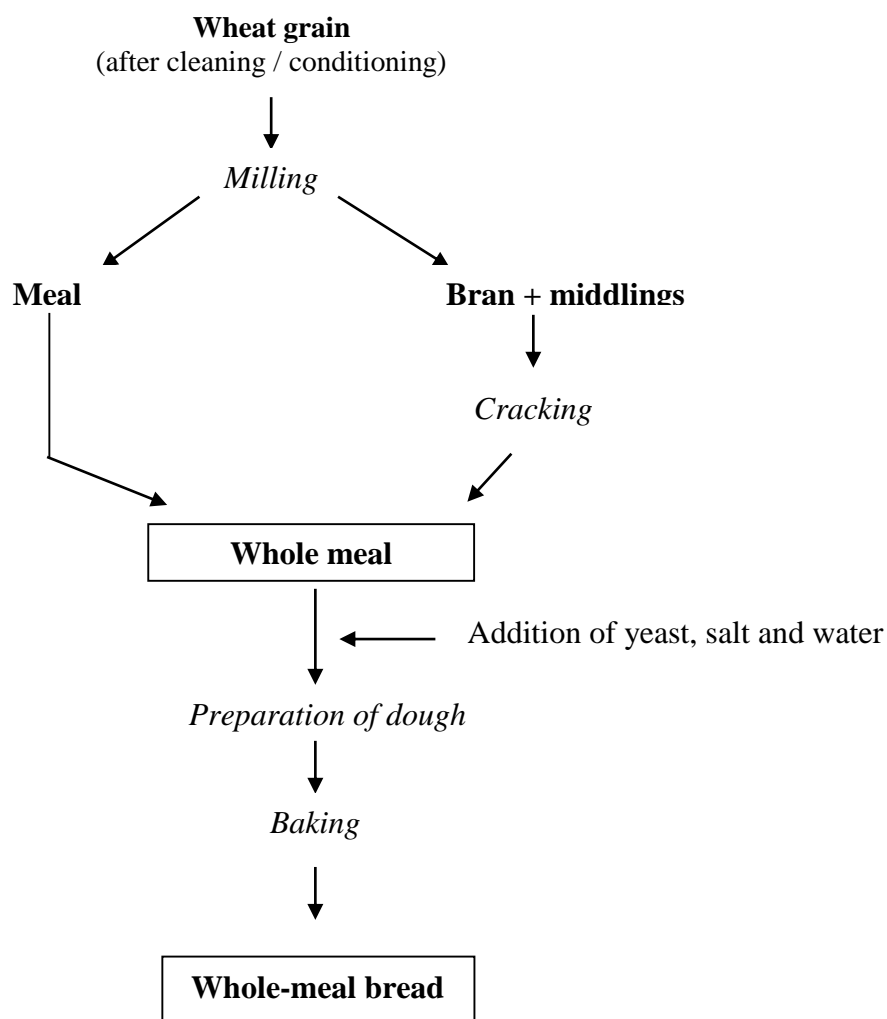
#### Production of Starch and Gluten

The first step of the production of starch and gluten was milling the grain to straight flour, bran and middlings. Straight flour and water were mixed to obtain a hydrated dough. The dough was separated by centrifugation into wet starch, process water and gluten (containing starch). Subsequently, the starch was washed out with water 3 times and separated by centrifugation into starch A, process water and gluten. Starch A was dried at 60°C and milled yielding the sample material starch A.

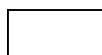
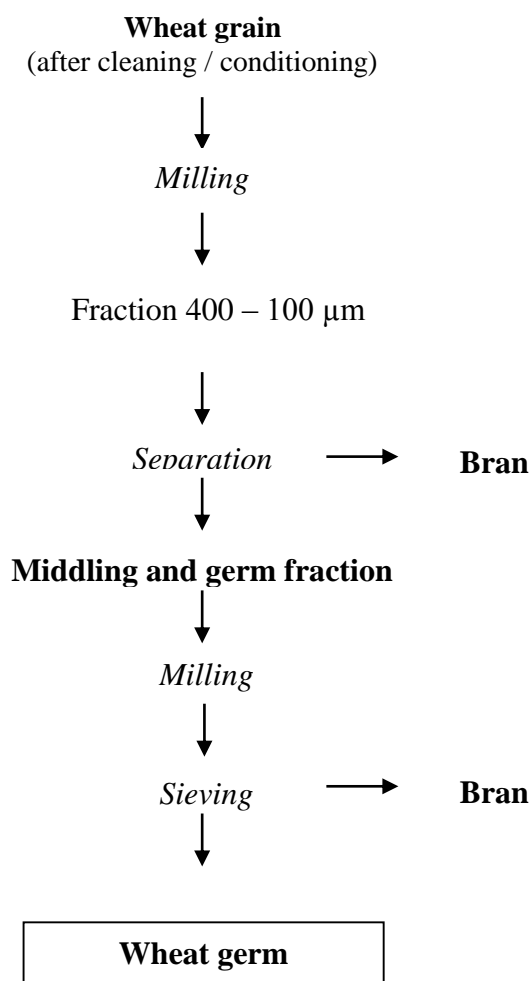
The gluten (containing starch) was washed several times out with water and resulted in gluten and process water (containing starch B and fibre). Gluten was dried by freeze drying, milled and sampled.

Remaining process water was separated by centrifugation into starch B, fibre and water. The sample materials starch B and fibre were dried at 60 °C, milled and collected. Milled starch B, gluten and fibre were combined to the sample material gluten feed meal.

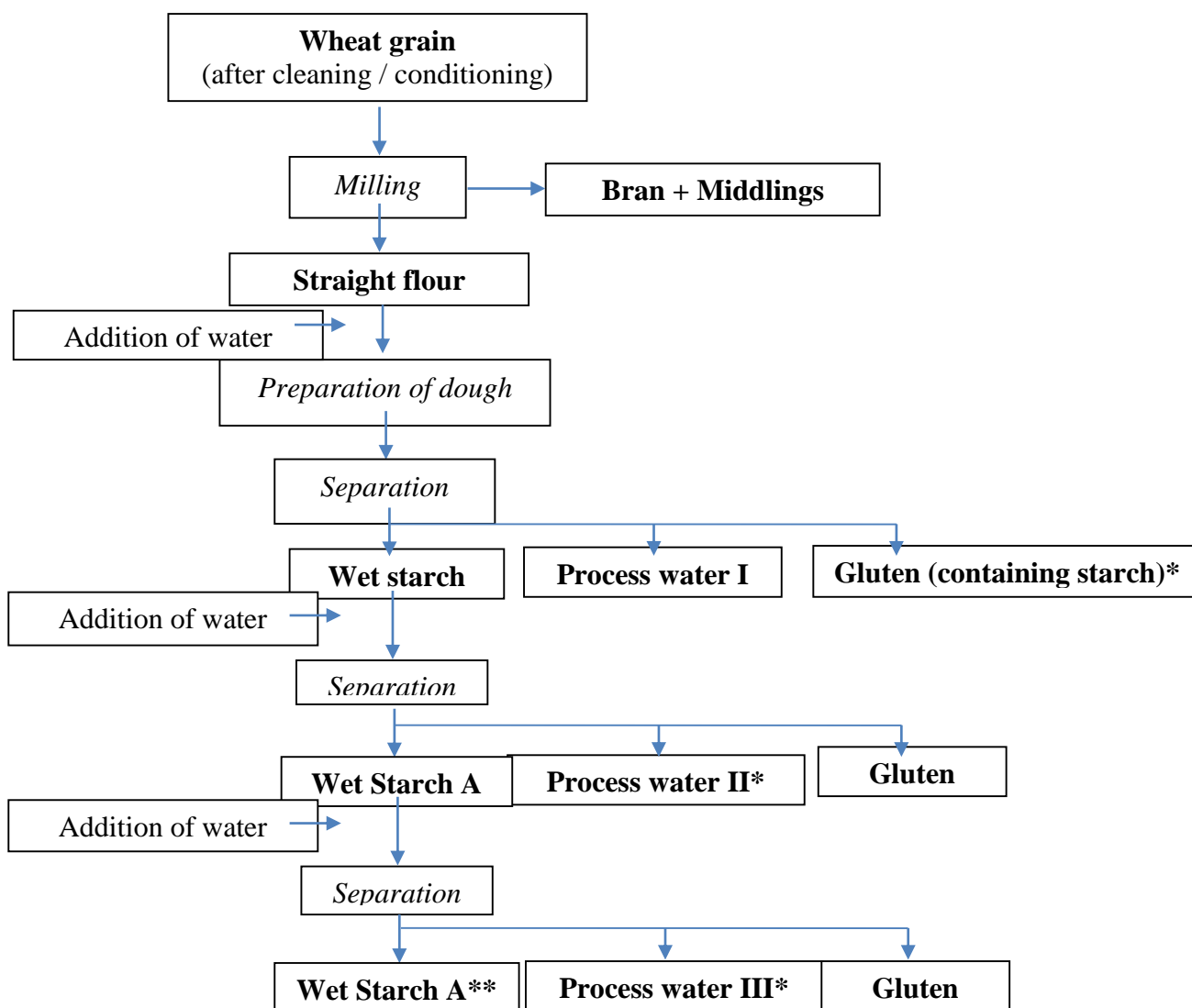
**Flow Charts****Figure 7.5.3.2-1: Milling of White Flour (Type 550) and Baking of White Bread**

**Figure 7.5.3.2-2: Milling of Whole Meal and Baking of Whole-meal Bread**

Laboratory samples to be taken and analysed

**Figure 7.5.3.2-3: Production of Wheat germ**

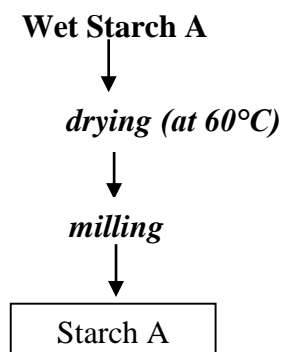
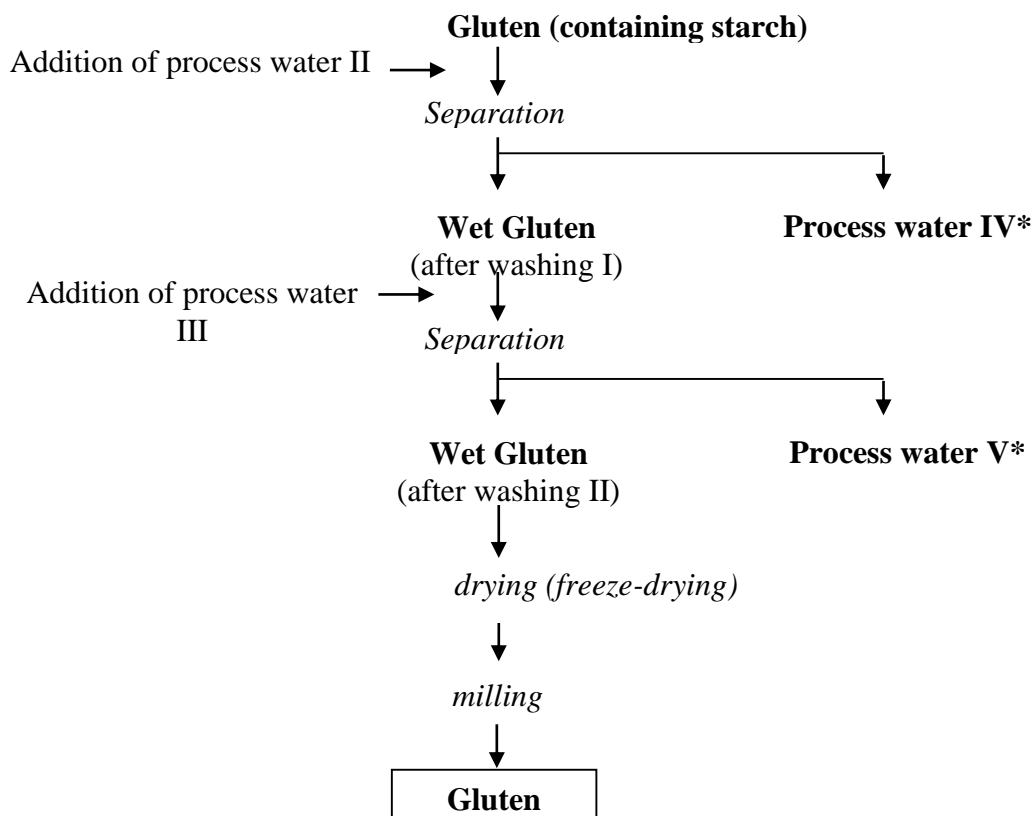
Laboratory samples to be taken and analysed

**Figure 7.5.3.2-4: Production of Starch and Gluten (general)**

\* used for further processing (see Fig 7.5.3.2-6)

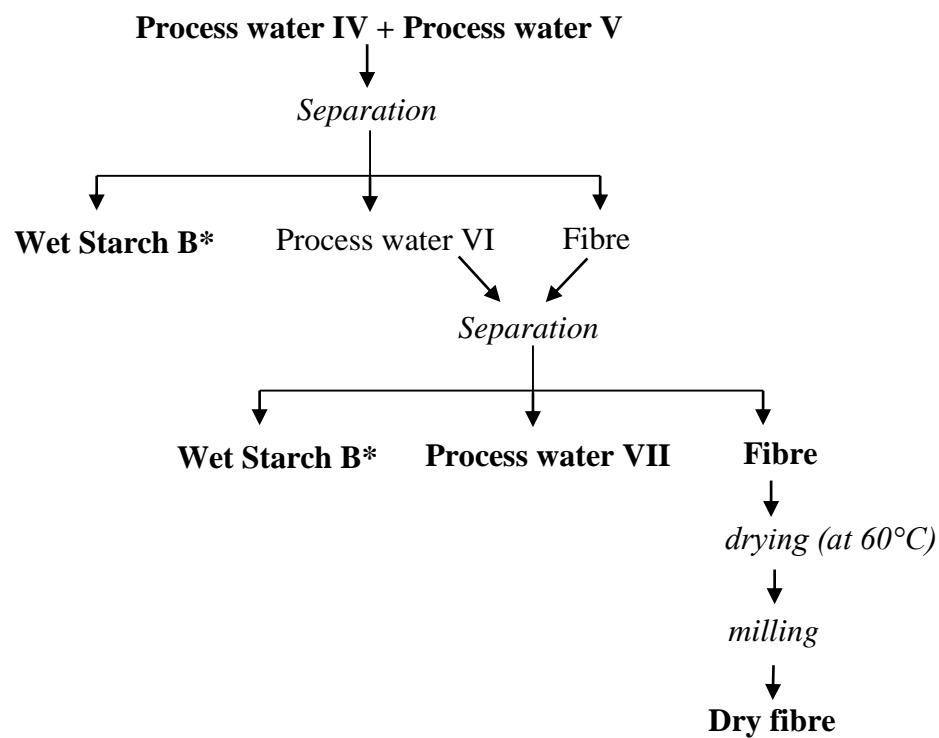
\*\* used for further processing (see Fig 7.5.3.2-5)

Laboratory samples to be taken and analysed

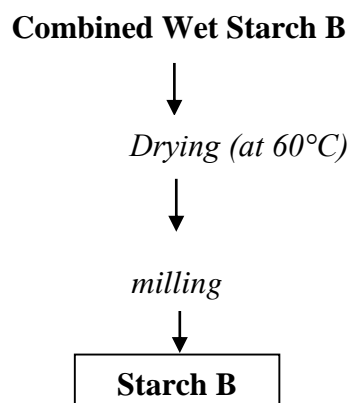
**Figure 7.5.3.2-5: Production of Starch A****Figure 7.5.3.2-6: Production of Gluten**

\* used for further processing steps see Fig 7.5.3.2-7.

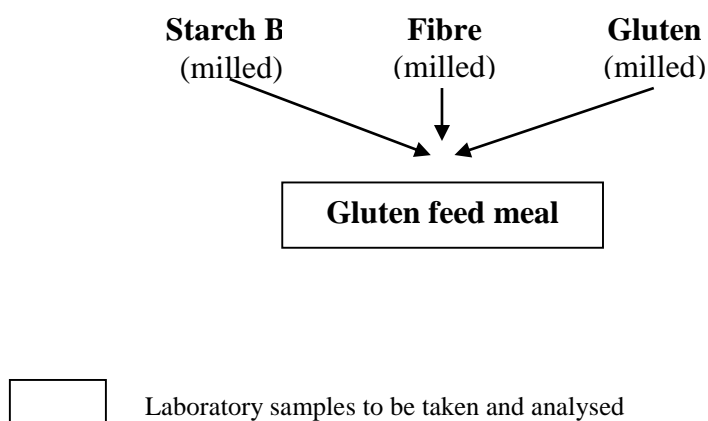
Laboratory samples to be taken and analysed

**Figure 7.5.3.2-7: Production of Wet Starch B and Dry fibre**

\* used for further processing steps see Figure 7.5.3.2-8.

**Figure 7.5.3.2-8: Production of Starch B**

Laboratory samples to be taken and analysed

**Figure 7.5.3.2-9: Production of Gluten feed meal****Findings**

Recovery rates were determined prior to analysis in order to validate the method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. The sample materials chosen served to represent all relevant sample materials collected in this study. The data demonstrate acceptable method performance during sample analysis. The summaries of recoveries are provided in Table 7.5.3.2-1. No residues were determined in the control samples.

Residues in wheat grain and the processed fractions are summarised in Table 7.5.3.2-1 and in more detail in the Appendix 1.

In the grain samples taken at harvest, flufenacet residues amounted to 0.1/0.085 mg/kg and 0.011/0.015 mg/kg (double sampling) for both trials. Processing factors were calculated based on the mean values from the individual studies.

For all processed commodities, transfer factors were calculated since residue levels above the LOQ were measured in the raw agricultural commodities, even though for some processed commodities the residue levels were less than the LOQ. In such cases the residue level was set at the LOQ in order to calculate a transfer factor. However, only cases in which both the raw agricultural commodity and the processed fraction show measurable residues are considered to truly indicate a processing factor (see Table 7.5.3.2-2). Processing factors are compiled in Table 7.5.3.2-3 for both trials.

**Storage period for samples:**

The storage period of grain field samples and processed samples ranged between 243 and 338 days. Samples were kept deep frozen at -18°C or below before processing starts and were returned to the freezer (-18°C) after termination of the processing until analysis.

All storage intervals are covered by the storage stability testing.

**Table 7.5.3.2-1: Recovery data for Flufenacet**

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./ metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
11-3401  11-3401-01 and 11-3401-02 GLP: yes 2011	Wheat, winter	grain	total residue flufenacet	1	<b>0.01</b>	89	89	89	89	--
				1	0.10	81	81	81	81	--
				2	overall		81	89	85	--
		white flour/ middlings <sup>a)</sup>	total residue flufenacet	3	<b>0.01</b>	107;111; 111	107	111	110	2.1
				4	0.10	95;109; 111;113	95	113	107	7.6
				7	overall		95	113	108	5.6
		white bread/ wholemeal bread	total residue flufenacet	2	0.10	86;87	86	87	87	
				2	overall		86	87	87	
		wheat germ	total residue flufenacet	3	<b>0.01</b>	90;95; 112	90	112	99	11.6
				3	0.10	88;90; 94	88	94	91	3.4
				6	overall		88	112	95	9.3
		gluten feed meal/ starch A <sup>b)</sup>	total residue flufenacet	3	<b>0.01</b>	80;89; 93	80	93	87	7.6
				4	0.10	86;87; 91;88	86	91	88	2.5
				7	overall		80	93	88	4.7

Fortified with flufenacet, determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet.

a) recoveries for white flour and middlings are also representative for shorts, whole meal flour and bran

b) recoveries for gluten feed meal and starch A are also representative for starch B and gluten

**Table 7.5.3.2-2: Results of processing trials conducted with Flufenacet WG 60 (containing (60 % flufenacet) on wheat**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
11-3401  11-3401-01 GLP: yes 2011	Wheat, winter Robigus (Winter wheat nabim Gp 3)	United Kingdom CB22 5EU Cam-bridge Europe, North	60 WG	1	0.48	0.24	25	grain	135	0.10
								bran	135	0.085
								middlings	135	0.41
								shorts	135	0.28
								white flour	135	0.41
								white bread	135	0.012
								whole meal	135	0.046
								wholemeal bread	135	0.10
								wheat germ	135	0.086
								starch A	135	0.11
								gluten	135	<0.01
								starch B	135	0.091
								gluten feed meal	135	0.020
11-3401  11-3401-02 GLP: yes 2011	Wheat, winter Tabasco	Netherlands 1175 KD Lijnden Europe, North	60 WG	1	0.48	0.16	25	grain	120	0.053
								bran	120	0.11
								middlings	120	0.015
								shorts	120	0.067
								white flour	120	0.042
								white bread	120	0.069
								whole meal	120	<0.01
								wholemeal bread	120	0.011
								wheat germ	120	0.017
								starch A	120	0.016
								gluten	120	0.021
								starch B	120	<0.01
								gluten feed meal	120	0.015
										<0.01

\*Residues for flufenacet determined as 4-fluoro-N-isopropylaniline and calculated as flufenacet

**Table 7.5.3.2-3: Summary of processing factors for flufenacet in wheat processed fractions**

Commodity	Trial 11-3401-01 United Kingdom	Trial 11-3401-02 The Netherlands
Bran	4.4	5.2
Middlings	3.0	3.2
Shorts	4.4	5.3
White flour	0.1	< 0.8*
White bread	0.5	0.8
Whole meal	1.1	1.3
Wholemeal bread	0.9	1.2
Wheat germ	1.2	1.6
Starch A	< 0.1*	< 0.8*
Gluten	1.0	1.2
Starch B	0.2	< 0.8*
Gluten feed meal	0.6	< 0.8*

For the calculation of the processing factors the mean value of the residues in two RAC samples was used.

\* In case the residue level in the processed fraction was less than the LOQ, the LOQ was used for calculation of the transfer factor.

<b>Report:</b>	<b>KCA 6.5.3/03, Krolski, M. E.; 1997; M-002403-01_</b>
Title:	FOE 5043 60 DF - Magnitude of the residue in wheat processed commodities and aspirated grain fractions
Document No	<b>M-002403-01-1</b>
Report No	107840 dated 1997-12-03
Guidelines:	EPA Ref.: 860.1500, 860.1520
GLP	Yes ; Deviations : none

### Materials and methods

In one trial conducted in Stilwell, Kansas (NAFTA Region 5) during the 1995 growing season, flufenacet was applied as FOE 5043 60 DF (60% DF flufenacet formulation) once with an early post-emergence foliar application (BBCH 14) to winter wheat at 2017 g ai/ha (8.4N). One treated and one untreated control bulk wheat grain sample was harvested at normal maturity, 115 days after treatment. The wheat grain samples were processed into bran, flour, shorts, middlings, and germ; aspirated grain fractions were also collected. All of the procedures simulated commercial wheat processing practices.

Residues of the raw agricultural commodity and the processed fractions were analysed using method 00346 (Seym, M.; 1995; M-018864-02 ) reported previously (Annex II dossier) which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues were determined using GC-MSD with an LOQ of 0.05 mg/kg and are expressed as parent flufenacet.

#### Processing procedures

##### Aspirated grain fractions

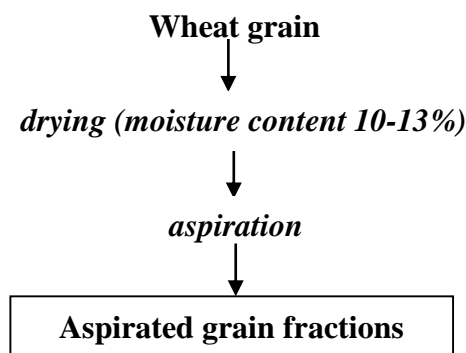
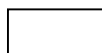
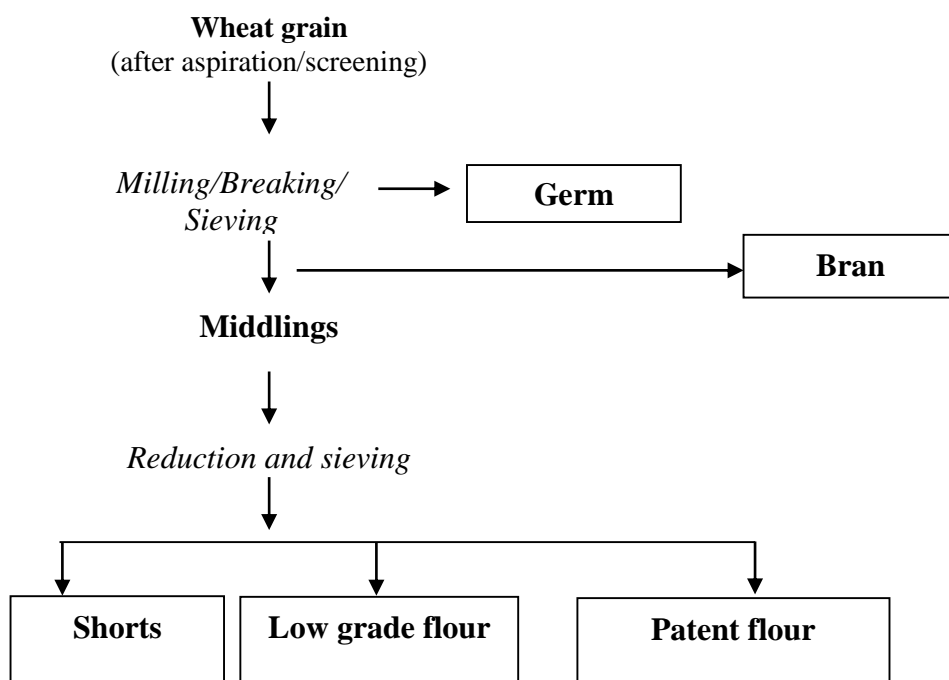
After determining the moisture content of the grain, the sample was dried in an oven until the moisture content was 10-13%. After drying, the sample was placed in a dust generation room containing holding bins, drag conveyors, and a bucket conveyor. As the sample was moved in the system, aspiration was used to remove light impurities (grain dust). The light impurities were then classified by sieving. Drying used for dust generation took precedence over drying before processing, and the light impurities collected during generation were kept separately from those collected during cleaning before processing.

##### Preparation of germs

The samples were aspirated and screened to separate light impurities and screenings (small and large) from the wheat. For wheat germ recovery, the cleaned wheat was moisture adjusted to 16% (1 to 1.5 hours), milled, and sifted to separate the bran from the germ fraction. The germ (with endosperm) was then passed through a reduction mill and sifted to separate the germ from the endosperm.

##### Preparation of bran, flour, shorts and middlings

For flour, the cleaned wheat grain was moisture adjusted to 16% and broken four times in corrugated roller mills and sieved. After four breaks, material on top of the 730 µm sieve was collected as bran, material on top of the 390 and 240 µm screens was combined as middlings, material on top of the 132 µm screen was considered low grade flour, and material through the 132 µm screen was patent flour. After bran separation, the middlings were reduced four times into flour with a smooth roller mill and sieved. After the fourth reduction, material again was separated corresponding to particle size into shorts, low grade flour, and patent flour. Low grade flour and patent flour from the reducing steps were combined with the flours from the break steps.

**Flow Charts****Figure 7.5.3.2-10: Generation of aspirated grain fraction****Figure 7.5.3.2-11: Generation of germ, bran, middlings, shorts, low grade flour and patent flour**

Laboratory samples to be taken and analysed

**Findings**

Recovery rates were determined prior to analysis and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Validation of processed fractions was conducted using the parent compound and metabolites containing the *N*-fluophenyl-*N*-isopropyl functional group. For flufenacet, fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding relative standard deviations (RSD) in grain

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and processed fractions were satisfactory, as shown in Table 7.5.3.2-4. The sample materials chosen served to represent all relevant sample materials collected in this study. No residues were determined in the control samples.

Residues in wheat grain and the processed fractions are summarised in Table 7.5.3.2-5 and in more detail in the Appendix 1.

In the grain samples taken at harvest, flufenacet residues amounted to 1.76 mg/kg (mean of 3 individual samples). For all processed commodities 3 individual samples were analysed and processing factors were calculated based on the mean per commodity. Processing factors are compiled in table 7.5.3.2-6.

Storage interval of samples

Grain field samples were stored frozen up to 25 months (766 days) and less than 1 months for processed fractions. The storage intervals are covered by the storage stability data.

**Table 7.5.3.2-4: Recovery data for Flufenacet** (the LOQ is marked in bold).

Study Trial No. Plot No.	Crop	Portion analysed	a.s./ metabolite*	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
107840  STF-F3082-94P GLP: yes 1995	Wheat winter	grain	total residue flufenacet	2	<b>0.05</b>	108;96	96	108	102	
				3	2.0	73;76;82	73	82	77	6.0
				5	overall		73	108	87	16.9
		bran	total residue flufenacet	13	<b>0.05</b>	108;112;116;106;110;102;88;86;86;98;98;100;84	84	116	100	10.9
				3	5.0	84;86;94	84	94	88	6.0
				16	overall		84	116	97	11.2
		flour	total residue flufenacet	13	<b>0.05</b>	102;96;100;100;114;92;92;84;90;80;86;88;88	80	114	93	9.7
				3	1.0	115;89;90	89	115	98	15.0
				16	overall		80	115	94	10.5
		shorts	total residue flufenacet	13	<b>0.05</b>	88;94;102;86;114;114;90;76;86;82;84;80;90	76	114	91	13.1
				3	2.0	70;80;71	70	80	74	7.5
				16	overall		70	114	88	14.8
		middlings	total residue flufenacet	13	<b>0.05</b>	108;98;108;98;104;110;90;98;96;96;98;90;88	88	110	99	7.2
				3	2.0	93;83;70	70	93	82	14.1
				16	overall		70	110	96	10.6
		germ	total residue flufenacet	13	<b>0.05</b>	85;82;104;102;106;118;78;104;98;86;92;114;72	72	118	95	14.8
				3	5.0	83;78;91	78	91	84	7.8
				16	overall		72	118	93	14.7
		aspirated grain fractions	total residue flufenacet	6	<b>0.05</b>	74;76;98;98;82;108	74	108	89	15.6
				3	1.0	87;100;98	87	100	95	7.4
				9	overall		74	108	91	13.0

\* Spiking with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate hydrate, flufenacet sulfonic acid sodium salt, flufenacet thioglycolate sulfoxide. Residues for flufenacet determined as FOE 5043 trifluoroacetamide and calculated as flufenacet.

**Table 7.5.3.2-5: Results of processing trials conducted with Flufenacet 60 DF (containing 60 % flufenacet) on wheat**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	Growth stage	Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
107840 STF-F3082-94P GLP: yes 1995	Wheat, winter Karl 92	USA Stilwell, Kansas America, North	60 WG	1	2.016	2.1	4-leaf	grain	115	1.76
								bran	115	3.61
								flour	115	0.78
								shorts	115	1.56
								middlings	115	1.41
								germ	115	2.28
								aspirated grain fractions	115	0.86

\*Residues for flufenacet determined as FOE 5043 trifluoroacetamide and calculated as flufenacet.  
For grain and the processed fractions the mean of 3 individual samples was calculated.

**Table 7.5.3.2-6: Processing factors for flufenacet in wheat processed fractions**

Commodity	Average residue from 3 samples (mg/kg)	Processing factor (as given in the report)	Processing factor (calculated)
Bran	3.61	2.1	2.1
Flour	0.78	< 1	0.44
Shorts	1.56	< 1	0.89
Middlings	1.41	< 1	0.80
Germ	2.28	1.3	1.3
Aspirated grain fractions	0.86	< 1	0.49

The processing factor was calculated based on the mean value of 3 individual samples for the RAC and the processed fractions.

## Conclusion

Three processing trials on wheat are reported, two available from Europe and one study was performed in the US (see Table 7.5.3.2-7). Flufenacet was applied to the crop at exaggerated rates (2N and 8N) with a WG or DF formulation. Wheat samples were processed into commercially representative fractions and aspirated grain fractions were obtained from the US trial. Residues of flufenacet were found to concentrate in bran (median 4.4X), middlings (median 3.0X), shorts (median 4.4X), whole meal (mean 1.2X) and whole meal bread (mean 1.1X), germ (median 1.3X) and gluten (mean 1.1X). No concentration of flufenacet residues were seen in white flour, white bread, starch, gluten feed meal and aspirated grain fractions.

**Table 7.5.3.2-7: Summary of processing factors for flufenacet in wheat processed fractions**

Commodity	Report no 11-3401 Trial 11-3401-01 United Kingdom	Report no 11-3401 Trial 11-3401-02 The Netherlands	Report No 107840 Stilwell, Kansas (US)	Mean / Median processing factor*
Bran	4.4	5.2	2.1	4.4
Middlings	3.0	3.2	0.80	3.0
Shorts	4.4	5.3	0.89	4.4
White flour	0.1	< 0.8	0.44	0.3
White bread	0.5	0.8	--	0.7
Whole meal	1.1	1.3	--	1.2
Wholemeal bread	0.9	1.2	--	1.1
Wheat germ	1.2	1.6	1.3	1.3
Starch A	< 0.1	< 0.8	--	--
Gluten	1.0	1.2	--	1.1
Starch B	0.2	< 0.8	--	--
Gluten feed meal	0.6	< 0.8	--	--
Aspirated grain fractions	--	--	0.49	--

\*The median is given in case more than 2 individual results are available; in case of two individual results > LOQ the mean value is calculated.

## BARLEY

Report:	KCA 6.5.3/05, Noss, G.; 2014; M-468736-02-1
Title:	Determination of the residues of flufenacet in/on barley and the processed fractions from pearl barley processing and preparation of alcoholic beverages (malting, brewing, distillation) after spray application of Flufenacet WG 60 in Germany and Belgium
Document No Report No	M-468736-02-1 11-3400 dated 2014-01-07
Guidelines:	<ul style="list-style-type: none"> <li>Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC</li> <li>EC Guidance working document 7029/VI/95 rev.5 (1997-07-22)</li> <li>EC guidance working document 7035/VI/95 rev.5 (1997-07-22)</li> <li>OECD Guideline for the Testing of Chemicals, Magnitude of the Pesticide Residues in Processed Commodities, 508 (2008-10-03),</li> <li>US EPA OCSPP Guideline No. 860.1520</li> </ul>
GLP	Yes ; Deviations : none

### Materials and methods

Two studies were performed in 2011 on spring barley in Germany and Belgium in order to collect sample material for processing studies. The samples of barley (grain) to be processed were obtained after one post emergence spray application (BBCH 23/25) at exaggerated rate (2N = 0.48 kg as/ha) with Flufenacet WG 60, an WG formulation containing 60 % flufenacet. The higher rate was used in order to obtain appropriate residue levels in the raw agricultural commodity for derivation of processing factors.

Barley grain samples to be processed were sampled 114-116 days after treatment at growth stage BBCH 89. The processing of the barley samples into the processed fractions was representative for production of beverages i.e. malting, brewing, distillation (beer, brewer's grain, brewer's yeast, brewer's malt, dried distillers grain, fresh distillers grain, malt sprouts, hops draff) and production of pearl barley (pearl barley rub off, pearl barley). Processing was performed simulating industrial processes at a laboratory scale.

Residues of the raw agricultural commodity and the processed fractions were analysed using method 01100/M002 (Stuke, S.; Teubner, L.; 2013; M-448503-01) with an LOQ of 0.01 mg/kg which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet.

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### Processing Procedures

#### Malting (see Figure 7.5.3.2-12)

After cleaning and sieving the grain the steeping process was conducted as a combined wet and dry steeping in a special steeping vessel activating enzymes until germination begins. The final steeping degree was in the range of 43.2 to 43.5 %

During the intensive respiration of the germinating grain the steeped good was turned over continuously. After germination, the life processes are terminated by kilning. Kiln-drying was conducted in a dry chamber. The maximum temperature during the kiln-drying process was 80.0°C. After kiln-drying the germs were removed mechanically by a trimmer. Brewer's malt and malt sprouts were sampled immediately after end of malting. Until brewing (approx. 4 weeks malt rest) the malt was stored at room temperature.

#### Brewing (see Figure 7.5.3.2-13)

##### *Mashing*

Before mashing, the brewer's malt was dry milled in a special malt mill. The crushed malt was mixed with brew water according to a definite temperature time regime (mash program) in order to obtain the extract of good quality.

##### *Lautering: Wort extraction and separation*

After mash boiling, the wort was separated from the insoluble malt components (brewer's grain). The extract remaining in the brewer's grain was extracted by washing with hot water (first filter runnings). The wort separation was done using a refining vat. After separation, the brewer's grain was sampled.

##### *Wort boiling and conditioning*

After addition of hop pellets, the separated wort was boiled (about 90 min at normal pressure). In order to deactivate the malt enzymes, sterilize the wort, extract essential components of the hops, precipitate high molecular proteins and expel unwanted aromatic substances.

After boiling, the flocs (hops draff) were separated in a whirlpool causing the sludge to deposit on the bottom. For cooling and ventilating the wort, an intra-plant circulation was used. By adding oxygen (intra-plant circulation) the conditions for the start of the fermentation were prepared.

##### *Fermentation and maturation*

In the pilot plant the classical primary fermentation (low fermentation) was carried out in bottom fermentation containers. The fermentation temperature was 9 °C.

As soon as the extract content of the fermented young beer was 2 % higher than the final attenuation, the storing time began. Before maturation the young beer was cooled down. During the main fermentation the yeast deposits on the tank bottom and was sampled as brewer's yeast.

At the beginning of maturation the young beer was stored at room temperature (warm maturation to break down the diacetyl) in casks. Then the young beer was stored under pressure (approx. 0.7 - 1.8 bar) at 2 °C (cold maturation) for approx. 4 weeks.

The rack beer was filtered using a special filter combination. The final product beer was sampled.

#### Distillers grain production (see Figure 7.5.3.2-14)

##### *Mashing*

Barley grain was cleaned and subsequently milled into coarse meal. The coarse meal was homogeneously mixed with water according to a definite temperature time regime (mash program).

##### *Fermentation*

For the fermentation yeast was added to the produced mash. The fermentation duration was 4 days (23 – 25 °C) and was stopped at reaching of the final attenuation. The alcohol content was 5.0 – 7.7 % v/v.

##### *Distillation*

The fermented mash was transferred in a distillation vessel and slowly heated up until the distillation temperature was reached. After reaching of 80 °C the temperature was very slowly increased to 100 °C. Alcohol distillation was done until the alcohol content in the distillate decreased to approx. 3 % vol. The remaining distillers wash was separated into thin distillers wash and into thick distillers wash (distillers grain, fresh) by using a centrifuge or a press. Distillers grain, fresh was sampled. Subsequently the remaining thick distillers wash was dried at 38 °C until moisture content < 10 % was reached. Distillers grain, dried was sampled.

Pearl barley production (see Figure 7.5.3.2-15)

*Cleaning and Conditioning*

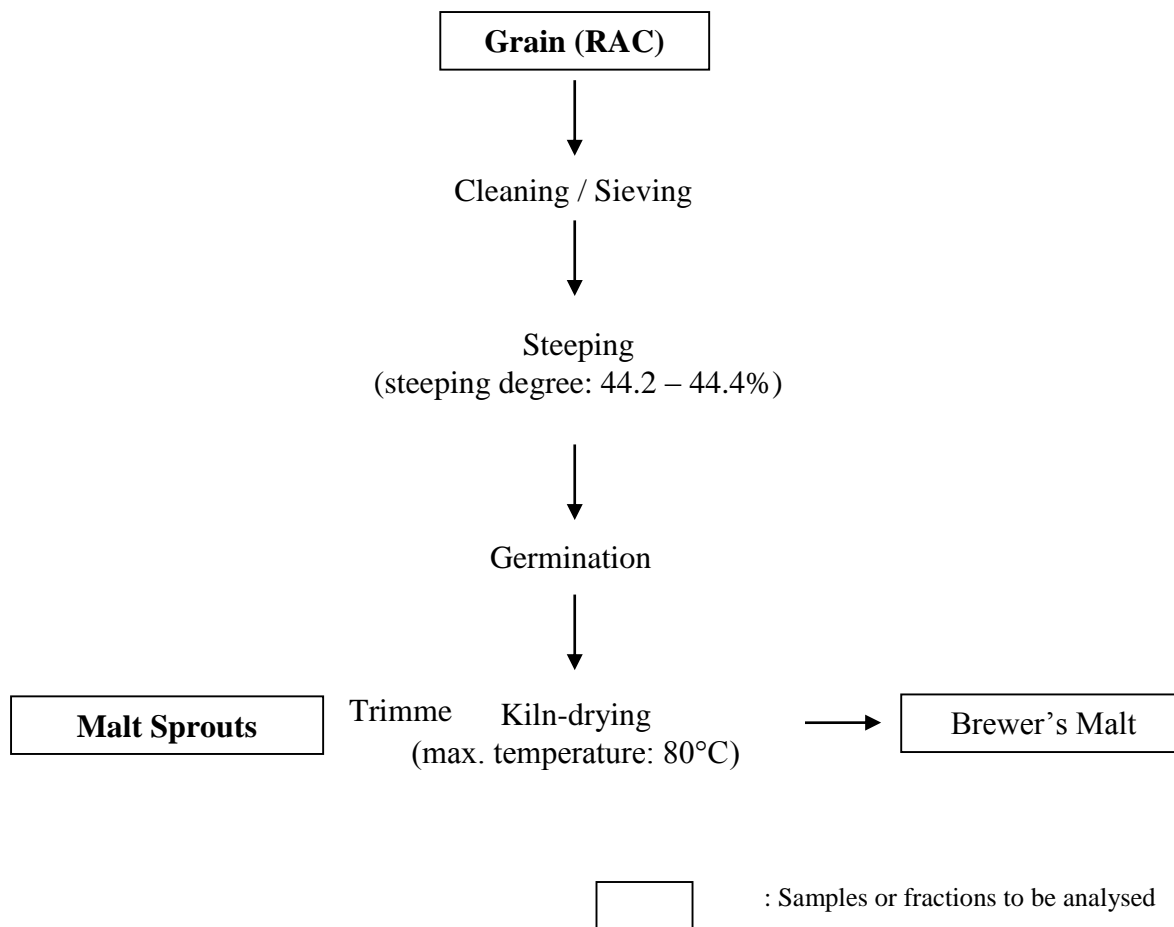
After cleaning barley grain was conditioned until an optimal moisture content of approx. 14 % was achieved.

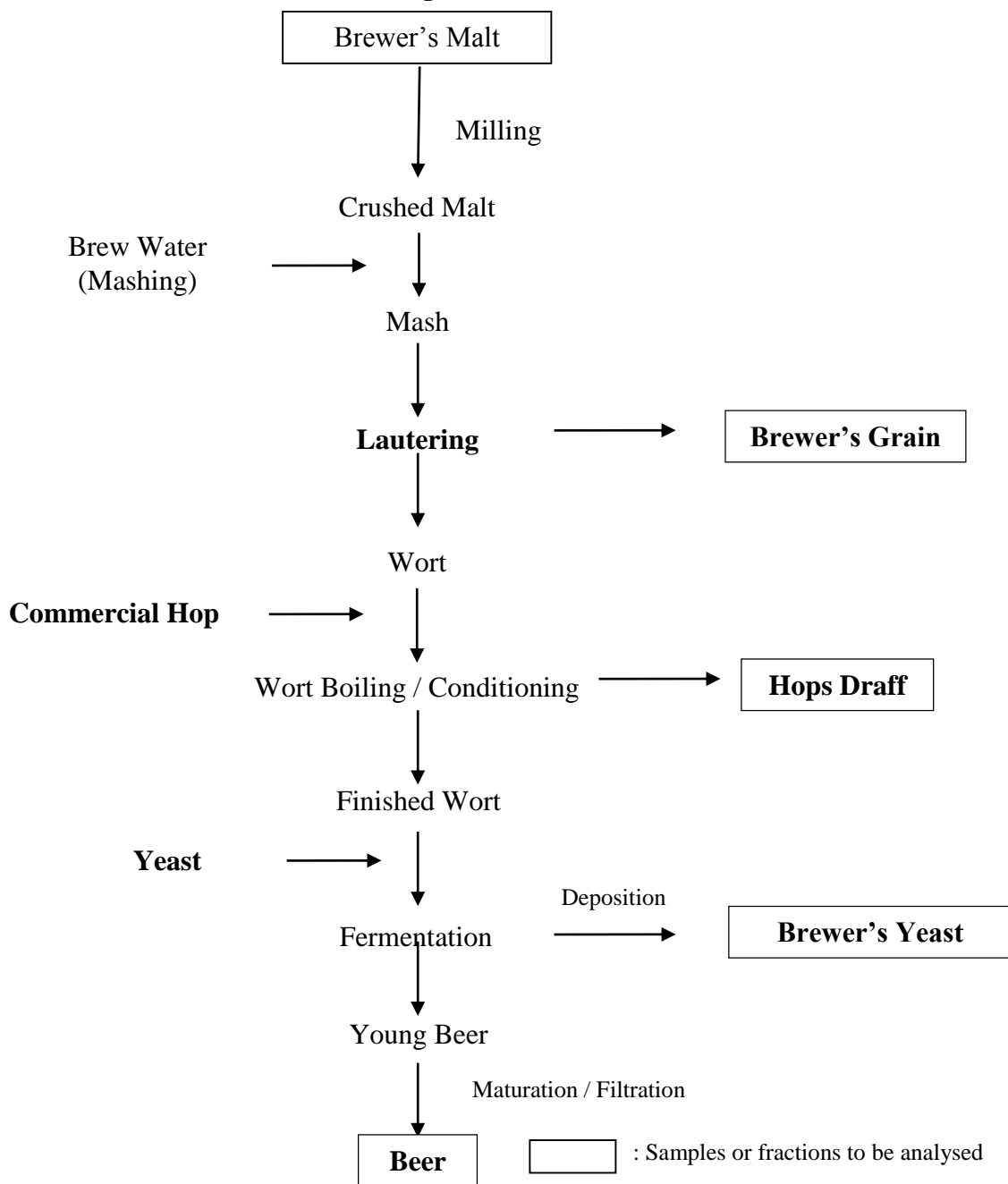
*Hulling*

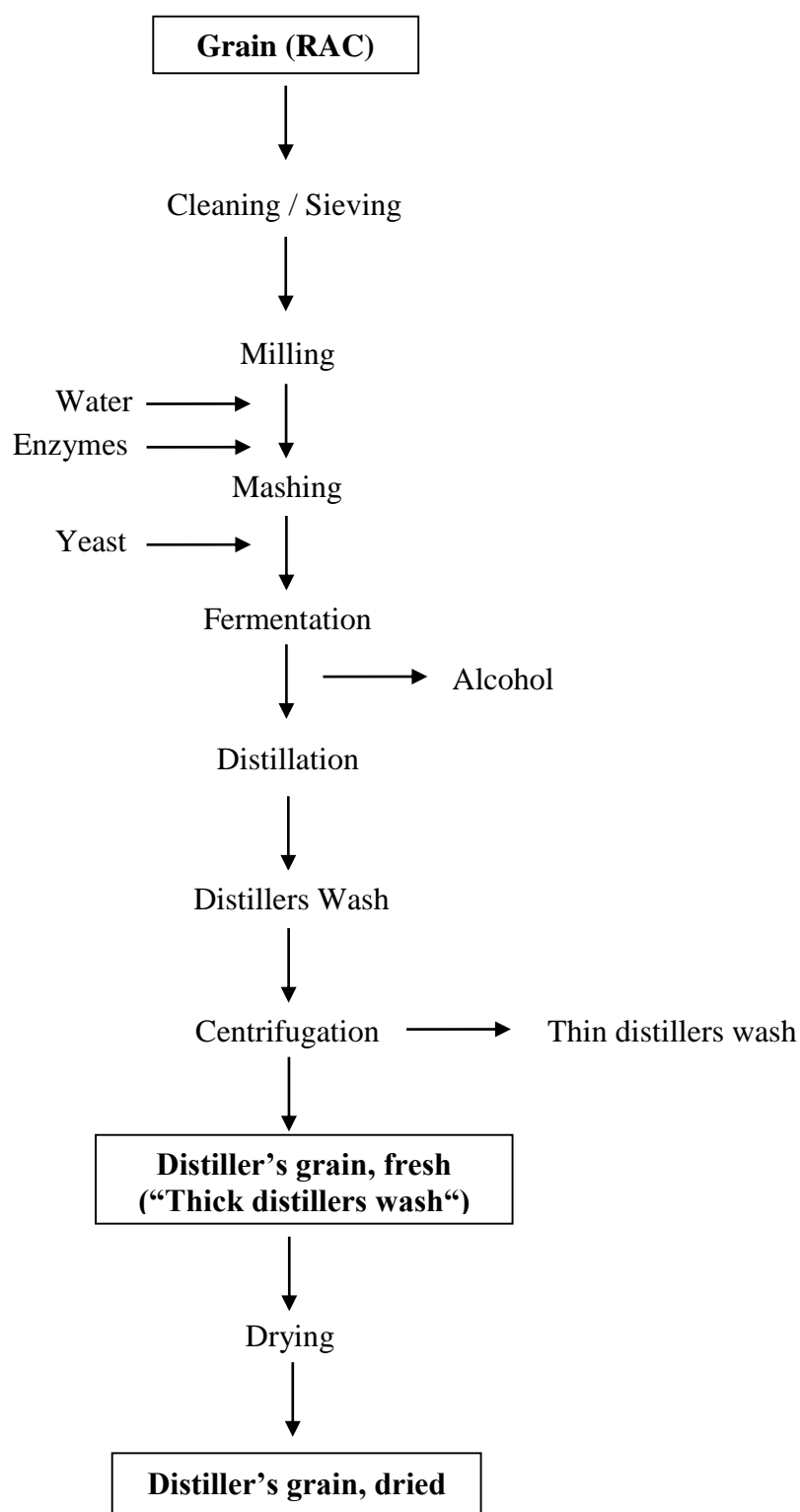
The corresponding samples were hulled until the stipulated abrasion for pearl barley (30 - 35 %) was reached. Pearl barley and pearl barley rub off were sampled.

**Flow Charts**

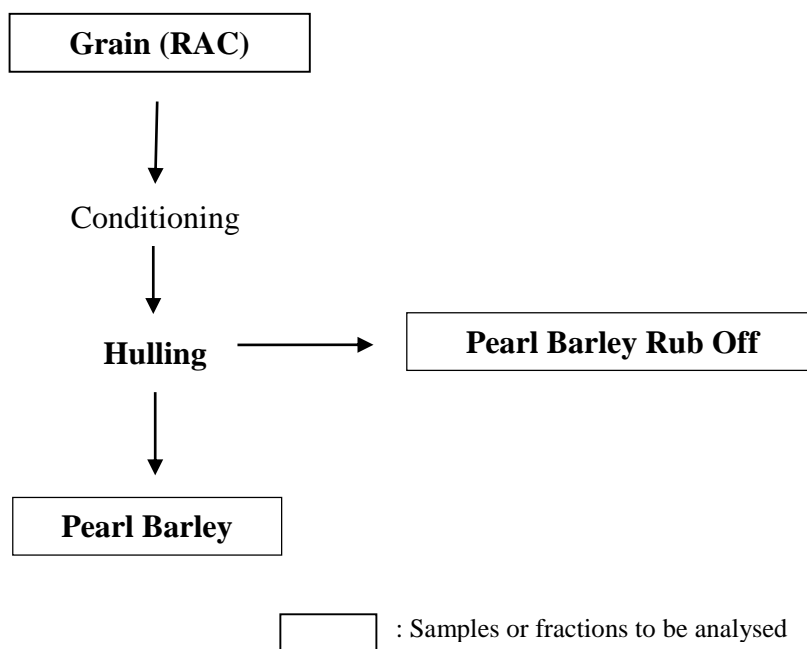
**Figure 7.5.3.2-12: Flow Chart of the Processing of Grain to Malt.**



**Figure 7.5.3.2-13: Flow Chart of the Processing of Malt to Beer.**

**Figure 7.5.3.2-14: Flow Chart of the Processing of Spring Barley to Distillers grain.**

: Samples or fractions to be analysed

**Figure 7.5.3.2-15: Flow Chart of the Processing of Spring Barley to Pearl Barley.**

### Findings

Recovery rates were determined prior to residue analysis in order to validate the method and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Control material was fortified with flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid and FOE5043 thioglycolate sulfoxide as a mixture (1/1/1/1). The sample materials chosen served to represent all relevant sample materials collected in this study. The data demonstrate acceptable method performance during sample analysis. The summaries of recoveries and corresponding relative standard deviations (RSD) are provided in Table 7.5.3.2-8. No residues were determined in the control samples.

Residues in barley grain and the processed fractions are summarised in Table 7.5.3.2-9 and in more detail in the Appendix 1.

In spite of the exaggerated rate used and late application during tillering in spring (BBCH 23/25) no flufenacet residues were determined in the raw agricultural commodity in both trials. Nevertheless grain was processed in order to investigate possible concentration. Except for the by-products destiller's grain (dried) and pearl barley rub off, residues were less than the LOQ also in processed fractions of barley. Thus, processing factors were calculated only for these commodities using the LOQ as residue level for the RAC. An overview on processing factors is compiled in table 7.5.3.2-10 for both trials.

### Storage period for samples:

Barley grain was stored at ambient temperature for 6-8 weeks until processing according to industrial practice in order not to compromise the germination processes by freezing the raw agricultural commodity. The storage period of deep-frozen laboratory samples intended for the analysis of flufenacet ranged between 12 and 21 months (350 - 619 days). The storage period is covered by the storage stability data.

**Table 7.5.3.2-8: Recovery data for Flufenacet**

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
11-3400MAN  11-3400-01 and 11-3400-02  GLP: yes 2011	Barley, spring	brewer's malt <sup>a)</sup>	Total residue flufenacet	4	<b>0.010</b>	91; 101; 107; 113	91	113	103	9.1
				3	0.10	72; 74; 74	72	74	73	1.6
				7	overall		72	113	90	19.1
		brewer's grain <sup>b)</sup>	Total residue flufenacet	5	<b>0.010</b>	75; 78; 79; 87; 105	75	105	85	14.3
				4	0.10	70; 73; 78; 100	70	100	80	16.9
				9	overall		70	105	83	14.7
		hops draff	Total residue flufenacet	3	<b>0.010</b>	89; 109; 117	89	117	105	13.7
				3	0.10	81; 99; 101	81	101	94	11.8
				6	overall		81	117	99	13.1
		brewer's yeast	Total residue flufenacet	4	<b>0.010</b>	86; 99; 106; 113	86	113	101	11.4
				3	0.10	75; 79; 94	75	94	83	12.1
				7	overall		75	113	93	15.0
		beer	Total residue flufenacet	4	<b>0.010</b>	63; 66; 68; 81	63	81	70	11.4
				3	0.10	64; 76; 77	64	77	72	10.0
				7	overall		63	81	71	10.1
		pearl barley <sup>c)</sup>	Total residue flufenacet	4	<b>0.010</b>	85; 91; 92; 97	85	97	91	5.4
				3	0.10	85; 86; 89	85	89	87	2.4
				7	overall		85	97	89	5.0
		grain, stored	Total residue flufenacet	5	<b>0.010</b>	62; 83; 89; 93; 101	62	101	86	17.2
				4	0.10	85; 87; 89; 91	85	91	88	2.9
				9	overall		62	101	87	12.2

RSD = Relative standard deviation, LOQ = Practical limit of quantification

Fortified with flufenacet, FOE5043 oxalate hydrate, FOE5043 sulfonic acid and FOE5043 thioglycolate sulfoxide (1/1/1/1), determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet<sup>a)</sup> Recoveries for brewer's malt are also representative for malt sprouts<sup>b)</sup> Recoveries for brewer's grain are also representative for distiller's grain fresh and dried<sup>c)</sup> Recoveries for pearl barley are also representative for pearl barley rub-off

**Table 7.5.3.2-9: Results of processing trials conducted with Flufenacet WG 60 (containing 60% flufenacet) on barley**

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
11-3400MAN  11-3400-01 GLP: yes 2011	Barley, spring Simba	Germany 49377 Vechta- Lang- foerden Europe, North	60 WG	1	0.48	0.16	25	malt sprouts	116	<0.01
								brewer's malt	116	<0.01
								brewer's grain	116	<0.01
								hops draff	116	<0.01
								brewer's yeast	116	<0.01
								beer	116	<0.01
								distillers grain, fresh	116	<0.01
								distillers grain, dried	116	0.012
								pearl barley rub off	116	0.018
								pearl barley	116	<0.01
								grain, stored	116	<0.01
									116	<0.01
									116	<0.01
									116	<0.01
11-3400MAN  11-3400-02 GLP: yes 2011	Barley, spring Quench	Belgium 6210 Saint- Amand Europe, North	60 WG	1	0.480	0.16	23	malt sprouts	114	<0.01
								brewer's malt	114	<0.01
								brewer's grain	114	<0.01
								hops draff	114	<0.01
								brewer's yeast	114	<0.01
								beer	114	<0.01
								distillers grain, fresh	114	<0.01
								distillers grain, dried	114	0.013
								pearl barley rub off	114	0.021
								pearl barley	114	<0.01

Study Trial No. Plot No. GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.)	kg/hL (a.s.)	GS	Portion analysed	DALT (days)	Total residue flufenacet* (mg/kg)
								grain, stored	114 114 114 114 114 114	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01

\*Residues for flufenacet determined as 4-fluoro-*N*-isopropylaniline and calculated as flufenacet

**Table 7.5.3.2-10: Summary of processing factors for flufenacet, in barley processed fractions**

Commodity	Trial: 11-3400-01, Germany	Trial: 11-3400-02, Belgium
<b>Processing into beer</b>		
malt sprouts	n.c.	n.c.
brewer's malt	n.c.	n.c.
brewer's grain	n.c.	n.c.
hops draff	n.c.	n.c.
brewer's yeast	n.c.	n.c.
beer	n.c.	n.c.
<b>Processing into distillers grain</b>		
Distillers grain, fresh	n.c.	n.c.
Distillers grain dried	>1.2*	>1.3*
<b>Processing into pearl barley</b>		
pearl barley rub off	>1.8*	>2.1*
pearl barley	n.c.	n.c.

n.c. = not calculated because residues in the raw agricultural commodity and the processed fraction were < LOQ.

\*In case residues in the processed fraction were >LOQ the LOQ of the RAC was used to calculate the transfer factor.

## Conclusion

Two processing trials were conducted in Europe on spring barley at exaggerated rates (2N) in order to obtain processing factors for sample materials representative for production of alcoholic beverages (malting, brewing, distillation) and production of pearl barley. The final consumable products as well as a number of by-products were analysed for residues of flufenacet. In spite of using an exaggerated application rate and application in spring no flufenacet residues could be determined in the RAC. No residues were determined in the processed fractions either, except for the by-products dried distiller's grain and pearl barley rub-off. The processing factor is considered to be indicative because the LOQ of the RAC has been used for calculation of the processing factor.

Following harvest, barley grain was stored at ambient temperature for a short time frame until processing according to industrial practice. However, it is not appropriate to store the grain deep-frozen since this would adversely affect the germination of the grain. Handling of the harvested produce truly reflects commercial processes and is therefore considered adequate. It is concluded that processing of barley when treated with flufenacet has no relevance for the consumer risk assessment.

### B.7.6. RESIDUES IN ROTATIONAL CROPS

Table 7.6-1 gives an overview on the metabolism studies in rotational crops.

**Table 7.6- 1: Overview of all plant metabolism studies with  $^{14}\text{C}$ -labeled flufenacet in succeeding crops**

Study type	Crop	Application scenario	Label	Report	Submission	
					EU dossier, Annex II , Section 4, Point 6	Presented in supplementary dossier Section 4, Point 6
Confined rotational crop	Wheat, Kale, Turnips	soil application 900 g as/ha	[Fluorophenyl-UL- $^{14}\text{C}$	Lenz, M.F. McKinney, M.K. (1994) M-002369-01-1	KCA 6.6.1/02	-
	Wheat, Kale, Turnips	soil application 900 g as/ha	[Thiadiazole-2- $^{14}\text{C}$	Harlarnkar, P.P., Mennicke, E.J. (1995) M-002368-01-1	KCA 6.6.1/01	-
	Wheat, Swiss chard, Turnips	soil application 900 g as/ha	[Thiadiazole-5- $^{14}\text{C}$	Bongartz, R., Klankers, M. (2012) M-443538-01-1		KCA 6.6.1/03

#### Plant metabolism studies evaluated during Annex I submission and reconsidered for renewal of active substance approval

##### References

**Report:** Lenz, M.F., McKinney, M.K., Accumulation of [phenyl- $^{14}\text{C}$ ]FOE 5043 residues in confined rotational crops, Bayer Corporation, report no.: MR 106768 (M-002369-01-1) of 17.10 1994  
**Guideline:** EPA, Ref: 165-1, Confined Accumulation Studies on Rotational Crops  
**GLP:** yes (certified laboratory)

**Report:** Harlarnkar, P.P., Mennicke, E.J., Accumulation of [thiadiazole-2- $^{14}\text{C}$ ]FOE 5043 residues in confined rotational crops, Bayer Corporation, report no.: MR 106639 (M-002368-01-1) of 26.05 1995  
**Guideline:** EPA, Ref: 165-1, Confined Accumulation Studies on Rotational Crops  
**GLP:** yes (certified laboratory)

Confined rotational crop studies with flufenacet were conducted using the  $^{14}\text{C}$ -labelled test substance, the radiolabel being in the [fluorophenyl-UL- $^{14}\text{C}$ ] and in the [thiadiazole-2- $^{14}\text{C}$ ] -position. In these studies bare soil was treated with 0.9 kg a.s./ha and residues in succeeding turnips, kale and wheat were characterised.

In review of the existing MRLs for flufenacet (EFSA Journal 2012;10(4):2689) EFSA concluded above study:

*In the DAR it was concluded that after use of flufenacet according to the GAPs (...), no significant residues are expected in leafy or root crops grown in rotation with the primary crops. According to the confined rotational crop metabolism studies the only exception to this would be wheat. However an assessment of the results from field trials in cereals and maize (...) shows that no residues are detected in any trial, except in green material sampled within 40 days of application and therefore it was concluded in the DAR that the high residue levels seen in wheat were a consequence of the experimental design and do not reflect normal practice. Considering, also, that the application rate of flufenacet within the EU ranges between 0.15-0.6 kg a.s./ha it can be concluded that flufenacet residue levels in rotational commodities are not expected to exceed 0.01 mg/kg, provided flufenacet is applied in compliance with the GAPs (...).*

Description of this study evaluated by RMS (France) during initial submission is cited below.

*The results of the confined rotational crop studies demonstrate that the metabolic pattern after application of FOE 5043 (flufenacet) is similar in target crops and crops grown in rotation. No active ingredient was found and all metabolites are derived by the same metabolic pathway via glutathione and homogluthathione, which is common to all plant species. Although several additional compounds were only observed in rotational crops, they are considered as products of further metabolism of known metabolites. Most of them should be detectable with the total residue method developed for plant residue analysis and/or are considered of being of no relevance because they are not expected to appear in significant amounts. After normal agricultural use of FOE 5043 no significant residues are to be expected in leafy or root crops grown in rotation with the target crops, even at rates which are considerably higher than the highest recommended field application in Europe. According to the above mentioned studies the only exception would be wheat (which at the same time is also a target crop). However, a comparison with the results from field trials in cereals and maize at recommended application rates of 240 a.i./ha and 600 g a.i./ha (see Chapter 6.3) [of the AII dossier] reveals that no residues were detected. Therefore, it is concluded, that the high residue levels in the confined rotational crop study are a consequence of the experimental design and do not reflect normal practice relevant conditions. Consequently, a field rotational crop study is considered as not being necessary.*

Since the highest supported application rates evaluated for Annex I inclusion and particularly the critical GAP for cereals did not change the conclusions drawn in the Monograph and in the EFSA reasoned opinion are still considered valid.

Since labelling in the thiadiazole-5-position is missing a new study has been added to complete the nature of residue constituents originating from flufenacet in succeeding crops.

#### B.7.6.1. Metabolism in rotational crops

##### Additional confined rotational crop study with [thiadiazole-5-<sup>14</sup>C]flufenacet

<b>Report</b>	<b>KCA 6.6.1/03, Bongartz, R.; Klankers, M.; 2012; M-443538-01_</b>
<b>Title:</b>	Metabolism of [thiadiazole-5- <sup>14</sup> C]Flufenacet in Confined Rotational Crops
<b>Document No:</b>	M-443538-01-1
<b>Report No:</b>	EnSa-12-0535 dated 2012-11-29
<b>Guidelines:</b>	OECD guideline 502: Metabolism in Rotational Crops, adopted 8-January-2007, US EPA OCSPP Residue Chemistry Guideline OPPTS 860.1850
<b>GLP</b>	Yes; Deviations: none

#### Executive Summary

Following confined rotational crop studies with [trifluorophenyl-UL-<sup>14</sup>C] and [thiadiazole-2-<sup>14</sup>C]flufenacet a respective study was conducted with flufenacet radiolabelled in the C-5 position of the thiadiazole ring to complete the picture of all potential metabolic pathways in rotated crops. Therefore, [thiadiazole-5-<sup>14</sup>C]flufenacet was applied to bare soil at a use rate of approximately 900 g as/ha and wheat (cereal crop), turnip (root crop) and Swiss chard (leafy crop) were sown 30 days (1<sup>st</sup> rotation), 142 days (2<sup>nd</sup> rotation) and 317 days (3<sup>rd</sup> rotation) after application. The crops were cultivated and harvested according to agricultural practice.

The total radioactive residues (TRR) increased in wheat from the 1<sup>st</sup> to the 2<sup>nd</sup> rotation and followed by a decrease at the 3<sup>rd</sup> rotation, whereas TRR continually decreased in turnip and Swiss chard from the 1<sup>st</sup> to the 3<sup>rd</sup> rotation. Extraction of harvested crops with acetonitrile/water (8/2, v/v) was almost complete amounting to more than 93% of TRR. Radio-HPLC and radio-TLC of the extracts revealed that more than 80% of TRR consisted of radiolabelled trifluoroacetate (TFA, M45) in all crops accompanied by minor amounts of FOE-thiadone-glycoside (M25) and trifluoroethane sulfonic acid (M44).

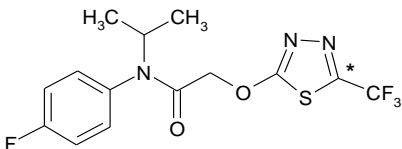
Soil core samples were taken shortly before each sowing. The residues in soil consisted mainly of the parent substance and TFA. FOE-thiadone (M9) was found at a minor extent. All residues in soil decreased with time.

These results indicated an initial cleavage of the thiadiazole ring from the parent substance in soil. Lower portions of the split-off thiadiazole ring were taken up by rotated crops and conjugated as glycoside. The main metabolic pathway proceeded via complete degradation of the thiadiazole ring in soil to form TFA (M45). On a short-term period, a low amount of trifluoroethane sulfonic acid (M44) was also formed in soil. The major portion of TFA and a small amount of the sulfonic acid obviously were taken up by the rotated crops since their concentration in the crops was higher than in the soil.

The proposed metabolic pathway of [thiadiazole-5-<sup>14</sup>C] in rotated crops is shown in Figure 7.6.1-1.

## Material and methods

### Test Material

Structural formula	 <p>* denotes the <sup>14</sup>C label</p>
Chemical name	<p><i>N</i>-(4-Fluoro-phenyl)-<i>N</i>-isopropyl-2-(5-trifluoromethyl-[1,3,4]thiadiazol-2-yloxy)-acetamide (IUPAC);</p> <p>Acetamide, <i>N</i>-(4-Fluorophenyl)-<i>N</i>-(1-methylethyl)-2- [[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]- (9CI; CAS)</p>
Common name	Flufenacet
CAS RN	142459-58-3
Empirical formula	C <sub>14</sub> H <sub>13</sub> F <sub>4</sub> N <sub>3</sub> O <sub>2</sub> S
Company code	FOE 5043
Molar mass (non-labelled)	363.34 g/mol
Label	[thiadiazole-5- <sup>14</sup> C]Flufenacet
Specific radioactivity	1.9 MBq/mg (used in the study; the original test substance had a specific radioactivity of 3.81 MBq/mg or 103.04 µCi/mg)
Radiochemical purity	>99% by TLC and HPLC (radio-detection)
Chemical purity	>99% by HPLC (UV detection at 210 nm)

### Test Plants

1 <sup>st</sup> Species (small grain)	Spring wheat
Variety	Thasos
Harvested commodities	Forage (BBCH 29), Hay (BBCH 75-83), Grain and straw (BBCH 89-92, maturity)
2 <sup>nd</sup> Species (root crops)	Turnip
Variety	Rondo
Harvested commodities	Roots and leaves (BBCH 45-49, maturity)
3 <sup>rd</sup> Species (leafy crops)	Swiss chard
Variety	Lukullus
Harvested commodities	Top plant (BBCH 45, intermediate and BBCH 49, maturity)

#### Preparation of the spray mixture and application

The original radiolabelled test substance was diluted with non-labelled flufenacet resulting in a specific radioactivity of 1.9 MBq/mg. Addition of a blank formulation yielded a SC 500 formulation with a concentration of the active substance of 42.4% (w/w). Addition of water finally resulted in the spray mixture of a volume of 104.5 mL.

A plant container (surface area 1 m<sup>2</sup>) was filled with a sandy loam soil (67% sand, 18% silt, 15% clay, 1.2% organic carbon, pH 6.9 (CaCl<sub>2</sub>)). During the first rotation, the plant container was placed in an open vegetation hall with natural temperatures and sunlight conditions, but protected from rain by a glass roof. The glass roof was opened during the sunshine periods and automatically closed during rainfall. During the second and third rotation, the container was moved into a greenhouse.

The spray solution was applied to the bare soil surface of the prepared plant container using a computer controlled track sprayer fitted with a flat jet nozzle. The actual application rate amounted to 903 g as/ha; it was higher by 7.5% than the maximum annual application rate of 840 g as/ha. The homogeneity of spray was proven by ten round filter papers (1.5 cm diameter) randomly placed onto the surface before application. The stability of the test substance in the spray mixture was demonstrated by radio-HPLC before and after application. After spraying the soil remained undisturbed until sowing for the first rotation (30 days). The soil was watered to maintain adequate soil moisture.

#### Sowing and cultivation of rotated crops

The rotated crops were sown at three intervals after application (plant back intervals, PBI):

First rotation: PBI = 30 days

Second rotation: PBI = 142 days

Third rotation: PBI = 317 days

Shortly before each sowing the upper soil layer (10 cm) was loosed and intensively mixed. Soil cores to a depth of 15 cm were sampled to investigate additionally the degradation of flufenacet in soil. Wheat was sown in 5 rows over 0.5 m<sup>2</sup>. Turnip was sown in 1 row over 0.25 m<sup>2</sup> and Swiss chard in 2 rows also over 0.25 m<sup>2</sup>. The crops were grown to maturity. After harvest of the previous set of crops the crops for next rotation were sown.

Fertilizing, watering and plant protection measures were performed according to agricultural practice. During the outdoor season (first rotation, April – September 2011) the mean temperatures amounted to 16 – 22°C and the mean sunshine periods to 83 – 231 hours/month. During the greenhouse season (second and third rotation, September 2011 – June 2012) the mean temperatures were 17 – 22°C. The crops were artificially irradiated with greenhouse lamps at 35 kLux during the day period (6.00 – 20.00 h).

#### Harvesting and processing of rotated crops

Wheat samples were taken at forage stage (BBCH 29, end of tillering), at hay stage (BBCH 75 – 83, grain content milky – early dough) and straw and grain at maturity (BBCH 89 – 92, grain hard to very hard). Immature top wheat plants were cut above the soil surface (roots remained in the soil), cut in small pieces and homogenized in liquid nitrogen with aid of a high speed stirrer (Polytron). Mature plants were manually separated in grain and straw (empty ears and chaff were added to the straw) before homogenization in liquid nitrogen. The homogenized samples were stored in freezers at approximately -18°C until analysis.

Turnips were completely sampled in the interval shortly before maturity (BBCH 45, 50% of expected root diameter reached) and full maturity (BBCH 49, expansion complete) and separated into roots and leaves. Roots and leaves were cut into slices and pieces, homogenized in liquid nitrogen and stored at approximately -18°C until analysis.

The green parts of Swiss chard were harvested as intermediated commodity (BBCH 45, 50% of leaf mass reached) and at maturity (BBCH 49, typical leaf mass reached). The roots remained in the soil. The sampled foliage was cut into pieces, homogenized in liquid nitrogen and stored at approximately -18°C.

#### Radioassaying, extraction and analysis of the plant samples

Radioassaying (measurement of the radioactivity) was conducted by liquid scintillation counting (LSC). The counting was repeated three times. Quenching was automatically compensated using an external standard. Solid samples were firstly combusted and the formed  $^{14}\text{CO}_2$  absorbed in an alkaline scintillation liquid. The limit of quantification (LOQ) was set to twice the background radioactivity for radioassaying of solid samples. Given the aliquot amount of combustion and the specific radioactivity used in this study the LOQ for radioassaying was 0.002 mg parent equivalents/kg (0.002 mg equ/kg).

Homogenized plant samples were extracted with acetonitrile/water (8/2, v/v, 3x) using a high speed stirrer (Polytron) followed by one extraction with pure acetonitrile. The radioactivity contents of the extracts and the remaining solids were numerically summarized to yield the total radioactive residues (TRR) of the original sample. The extracts were combined, concentrated and analyzed for the metabolite profile by radio-HPLC and radio-TLC (TLC only done for polar HPLC fractions).

Radio-HPLC was conducted on a RP18 column (250 x 4.6 mm, 5  $\mu\text{m}$  particle size) operated with a gradient mixture of water/formic acid (99/1, v/v) and acetonitrile/formic acid (99/1, v/v) at 40°C. The HPLC system was equipped with a UV detector (254 nm) and a radiomonitor with a glass scintillator (cell size 370  $\mu\text{L}$ ). Column recovery (98 – 101%) was proven by comparison of the eluted and injected radioactivity. The LOQ for HPLC determination was derived from the background noise and the smallest radio-peak of the respective sample. HPLC-LOQs for samples of the first and second rotation were in the range of 0.005 – 0.05 mg equ/kg.

One-dimensional radio-TLC was conducted on a silica gel TLC plates (20 x 20 cm, layer thickness 0.25 mm). Development of the spotted plates was performed with a solvent mixture consisted of ethyl acetate/2-propanol/ water/acetic acid (65/24/11/1, v/v/v/v) after chamber saturation. The radioactive spots on developed plates were visualized and quantified using a Bio-Imaging Analyzer. Non-labelled FOE-5043-sulfonic acid (2,2,2-trifluoroethane sulfonic acid) used as reference standard was stained with aqueous 0.1% 'Pinacryptol yellow' and visualized by extinction of the fluorescence dye of the plate under UV light.

The radioactivity in the isolated polar HPLC fraction of the wheat forage sample of the first rotation was identified by LC-MS as  $^{14}\text{C}$ -trifluoroacetate and was later used as radiolabelled reference standard in co-chromatography of the other samples. LC-MS was conducted on a combination of RP18-HPLC and an Orbitrap mass spectrometer using electro-spray for ionization. Non-labelled FOE 5043-sulfonic acid and FOE-thiadone (5-trifluoromethyl-1,2,4-thiadiazol-2(3*H*)-one) were used as additional reference standards for co-chromatography.

#### Extraction of soil samples

The soil core samples (0 – 15 cm layer) of each sampling interval (shortly before sowing of rotated crops) were mixed, homogenized and extracted with acetonitrile/water (1/1, v/v, 3x) using a high-speed stirrer. The combined extracts were concentrated and analyzed by radio-HPLC and radio-TLC together with the parent substance and the mentioned reference standards for co-chromatography.

### **Findings**

#### Total radioactive residues in rotated crops and soil

Total radioactive residues (TRR) in the agricultural commodities of the three rotated crop species are presented in Table 7.6.1-1. They increased in wheat from the first to second rotation following by a decrease at the third rotation. In contrast, TRR continuously decreased in turnip and Swiss chard from the first to the third rotation (except Swiss chard of intermediate growth stage).

For comparison, TRR in soil samples taken shortly before each sowing steadily decreased from the first to the last rotation: TRR in soil: 1<sup>st</sup> rotation: 0.638 mg equ/kg; 2<sup>nd</sup> rotation: 0.239 mg equ/kg; 3<sup>rd</sup> rotation: 0.104 mg equ/kg.

Extractability and identification of the extracted residues in rotated crops

The extraction of rotated crops with acetonitrile/water (4/1, v/v) and pure acetonitrile was almost complete accounting to 93.1 - 100% of TRR. In turn, the non-extractable residues ("post extraction solids", PES) ranged from 0 to maximum 6.9% of TRR (wheat grain of the 3<sup>rd</sup> rotation).

Reversed-phase radio-HPLC profiles of the extracts were performed immediately after extraction. Radiolabelled trifluoroacetate (M45, isolated from wheat forage of the first rotation and identified by HPLC-MS), radiolabelled FOE-thiadone-glycoside (M25, isolated in wheat metabolism study of [thiadiazole-5-<sup>14</sup>C]flufenacet) and non-labelled FOE-trifluoroethane sulfonic acid served as reference standards for co-chromatography.

The predominant portion of the radioactive residues extracted from all crops proved to be very polar as it was eluted in reversed phase HPLC as a radio-peak close to the dead volume. This peak showed sometimes a shoulder and seems to represent more than one metabolite. Therefore, the respective fraction was collected and additionally analyzed by radio-TLC on a straight phase silica gel plate. The mentioned radiolabelled reference standards were used for co-chromatography.

It turned out that nearly the complete portion of polar radioactive residues (83.6 - 99.9% of TRR) consisted of <sup>14</sup>C-trifluoroacetate (M45). FOE-thiadone-glycoside (M25) and FOE 5043-trifluoroethane sulfonic acid (M44) were detected at minor amounts (< 10% of TRR). The rate of identification of the radioactive residues in all rotated crops was very high accounting for ≥ 92.5% of TRR. The composition of the radioactive residues in crops rotated after application of [thiadiazole-5-<sup>14</sup>C]flufenacet to bare soil at a rate of approximately 900 g as/ha is presented in Table 7.6.1-2 (first rotation), Table 7.6.1-3 (second rotation) and Table 7.6.1-4 (third rotation). Metabolic pathway of [thiadiazole-5-<sup>14</sup>C]flufenacet in rotated crops is shown in Figure 7.6.1-1.

Storage stability of the radioactive residues of flufenacet on rotated crops

All crop samples were stored at temperature ≤ -18 °C immediately after sampling until extraction and analysis.

The samples of the first rotation were extracted within 12 days after sampling, at maximum, those of the second rotation within 8 days and those of the third rotation within one month after sampling. The earliest metabolite profiles (used for quantitation of metabolites) were obtained by radio-HPLC analysis within 4 days after extraction.

Approximately one year after sampling, repeated extraction and profiling of metabolites were performed from wheat straw, wheat grain and Swiss chard (at maturity) of the first rotation using identical analytical conditions. There were no differences between the metabolite profiles of the initial and repeated analysis. Therefore, it is concluded that the residues of flufenacet in the samples of rotated crops were stable for at least one year.

Extraction and identification of extracted residues in soil

Soil core samples taken shortly before sowing of rotated crops (days 30, 142 and 317 days after application of the radiolabelled substance) were analyzed for the composition of residues. These analyzes revealed a continuous decrease of the parent substance from 0.459 to 0.043 mg/kg and a similar decrease of the major metabolite trifluoroacetate (M45) from 0.162 mg equ/kg to 0.034 mg equ/kg. The minor metabolite FOE-thiadone (M9) was only detected in the first soil sample (30 days after application) amounting to 4.5% of TRR corresponding to 0.030 mg equ/kg. The non-extractable residues increased from 23.9% to 58.9% of TRR. The composition of residues in soil is presented in Table 7.6.1-5.

## Conclusion

Following application of [thiadiazole-5-<sup>14</sup>C]flufenacet to soil at a use rate of approximately 900 g as/ha wheat (cereal crop), turnip (root crop) and Swiss chard (leafy crop) were sown and rotated 30 days (1<sup>st</sup> rotation), 142 days (2<sup>nd</sup> rotation) and 317 days (3<sup>rd</sup> rotation). Extraction of rotated crops with acetonitrile/water (8/2, v/v) was almost complete amounting to more than 93% of TRR. Radio-HPLC and radio-TLC of the extracts revealed that more than 80% of TRR consisted of radiolabelled trifluoroacetate (TFA, M45) in all crops accompanied with minor amounts of FOE-thiadone-glycoside (M25) and trifluoroethane sulfonic acid (M44).

These results indicated an initial cleavage of the thiadiazole ring from the parent substance in soil. Low amounts of the split-off thiadiazole ring were taken up by rotated crops and conjugated as glycoside. The main metabolic pathway proceeded via complete degradation of this ring in soil to form TFA (M45). On a short-term period, low amounts of trifluoroethane sulfonic acid (M44) were also found in soil. The major portion of TFA (M45) and a small amount of the sulfonic acid (M44) obviously was taken up by the rotated crops since their concentration in the crops were higher than in the soil.

The proposed metabolic pathway of [thiadiazole-5-<sup>14</sup>C] in rotated crops is shown in Figure 7.6.1-1.

**Table 7.6.1-1: Total radioactive residues (TRR) in rotated crops following application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 900 g as/ha to bare soil**

TRR in rotated crops	1 <sup>st</sup> rotation	2 <sup>nd</sup> rotation	3 <sup>rd</sup> rotation
PBI (days)	30	142	317
Crop commodity	[mg equ/kg]		
wheat forage	1.543	2.318	1.441
wheat hay	3.755	8.225	3.740
wheat straw	4.376	9.335	4.035
wheat grain	3.024	7.673	1.371
turnip leaves	6.792	3.536	0.993
turnip roots	0.601	0.197	0.087
Swiss chard (intermediate)	6.117	1.951	4.784
Swiss chard (at maturity)	3.386	2.950	1.973

**Table 7.6.1-2: Composition of the radioactive residues in crops of the 1<sup>st</sup> rotation after application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 900 g as/ha to bare soil**

Wheat, 1 <sup>st</sup> rotation	Forage		Hay		Straw		Grain	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	95.2	1.469	90.7	3.404	92.6	4.054	95.9	2.899
FOE 5043-trifluoroethane sulfonic acid	3.3	0.50	2.1	0.079	0.5	0.021	n.d.	n.d.
FOE-thiadone-glycoside	1.3	0.019	3.8	0.142	4.5	0.198	n.d.	n.d.
<b>Total identified</b>	<b>99.7</b>	<b>1.538</b>	<b>96.6</b>	<b>3.625</b>	<b>97.7</b>	<b>4.274</b>	<b>95.9</b>	<b>2.899</b>
unknown	n.d.***	n.d.	0.4	0.016	0.6	0.027	n.d.	n.d.
Total characterized*	n.d.	n.d.	0.4	0.016	0.6	0.027	n.d.	n.d.
Procedural loss	---	---	---	---	0.2	0.008	3.5	0.106
<b>Total extractable</b>	<b>99.7</b>	<b>1.538</b>	<b>97.0</b>	<b>3.641</b>	<b>98.5</b>	<b>4.309</b>	<b>99.4</b>	<b>3.006</b>
Non-extractable (PES) **	0.3	0.004	3.0	0.113	1.5	0.067	0.6	0.018
<b>Accountability</b>	<b>100.0</b>	<b>1.543</b>	<b>100.0</b>	<b>3.755</b>	<b>100.0</b>	<b>4.376</b>	<b>100.0</b>	<b>3.024</b>

Turnip and Swiss chard 1 <sup>st</sup> rotation	Turnip leaves		Turnip roots		Swiss chard		Swiss chard	
	mature		mature		intermediate		mature	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	94.7	6.432	83.6	0.503	92.0	5.625	93.4	3.162
FOE 5043-trifluoroethane sulfonic acid	2.2	0.146	n.d.	n.d.	3.2	0.193	2.2	0.076
FOE-thiadone-glycoside	2.4	0.163	8.9	0.054	0.8	0.051	1.6	0.053
<b>Total identified</b>	<b>99.3</b>	<b>6.741</b>	<b>92.5</b>	<b>0.556</b>	<b>95.9</b>	<b>5.870</b>	<b>97.2</b>	<b>3.291</b>
unknown	0.5	0.031	5.2	0.031	3.5	0.217	2.5	0.083
Total characterized*	0.5	0.031	5.2	0.031	3.5	0.217	2.5	0.083
Procedural loss	-	-	-	-	0.2	0.010	-	-
<b>Total extractable</b>	<b>99.7</b>	<b>6.772</b>	<b>97.8</b>	<b>0.588</b>	<b>99.7</b>	<b>6.096</b>	<b>99.7</b>	<b>3.374</b>
Non-extractable (PES) **	0.3	0.019	2.2	0.013	0.3	0.021	0.3	0.011
<b>Accountability</b>	<b>100.0</b>	<b>6.792</b>	<b>100.0</b>	<b>0.601</b>	<b>100.0</b>	<b>6.117</b>	<b>100.0</b>	<b>3.386</b>

\* unidentified compounds are characterized by their extraction and chromatographic behaviour.

\*\* PES = post extraction solids

\*\*\* n.d. = not detected

# mg/kg means mg parent equivalents/kg

**Table 7.6.1-3: Composition of the radioactive residues in crops of the 2<sup>nd</sup> rotation after application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 900 g as/ha to bare soil**

Wheat, 2 <sup>nd</sup> rotation	Forage		Hay		Straw		Grain	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	99.3	2.302	99.7	8.198	98.6	9.205	99.4	7.624
FOE-thiadone-glycoside	0.6	0.014	n.d.**	n.d.	0.5	0.051	n.d.	n.d.
Total identified	99.9	2.316	99.7	8.198	99.2	9.256	99.4	7.624
Procedural loss	-	-	-	-	-	-	0.3	0.020
<b>Total extractable</b>	<b>99.9</b>	<b>2.316</b>	<b>99.7</b>	<b>8.198</b>	<b>99.2</b>	<b>9.256</b>	<b>99.6</b>	<b>7.643</b>
Non-extractable (PES) *	0.1	0.002	0.3	0.028	0.8	0.078	0.4	0.029
<b>Accountability</b>	<b>100.0</b>	<b>2.318</b>	<b>100.0</b>	<b>8.225</b>	<b>100.0</b>	<b>9.335</b>	<b>100.0</b>	<b>7.673</b>

Turnip and Swiss chard, 2 <sup>nd</sup> rotation	Turnip leaves		Turnip roots		Swiss chard		Swiss chard	
	mature		mature		intermediate		mature	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA	99.9	3.534	97.0	0.191	100.0	1.951	99.8	2.946
FOE-thiadone-	n.d.**	n.d.	2.6	0.005	n.d.	n.d.	n.d.	n.d.
<b>Total identified</b>	<b>99.9</b>	<b>3.534</b>	<b>99.5</b>	<b>0.196</b>	<b>100.0</b>	<b>1.951</b>	<b>99.8</b>	<b>2.946</b>
Procedural loss	-	-	-	-	-	-	-	-
<b>Total</b>	<b>99.9</b>	<b>3.534</b>	<b>99.5</b>	<b>0.196</b>	<b>100.0</b>	<b>1.951</b>	<b>99.8</b>	<b>2.946</b>
Non-extractable	0.1	0.003	0.5	0.001	<0.1	0.001	0.2	0.005
<b>Accountability</b>	<b>100.0</b>	<b>3.536</b>	<b>100.0</b>	<b>0.197</b>	<b>100.0</b>	<b>1.951</b>	<b>100.0</b>	<b>2.950</b>

\* PES = post extraction solids

\*\* n.d. = not detected

# mg/kg means mg parent equivalents/kg

**Table 7.6.1-4: Composition of the radioactive residues in crops of the 3<sup>rd</sup> rotation after application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 900 g as/ha to bare soil**

Wheat, 3 <sup>rd</sup> rotation	Forage		Hay		Straw		Grain	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA (trifluoroacetic acid)	99.9	1.440	99.7	3.729	99.2	4.004	93.1	1.277
FOE-thiadone-glycoside	n.d.**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Total identified</b>	<b>99.9</b>	<b>1.440</b>	<b>99.7</b>	<b>3.729</b>	<b>99.2</b>	<b>4.004</b>	<b>93.1</b>	<b>1.277</b>
Procedural loss	-	-	-	-	-	-	-	-
<b>Total extractable</b>	<b>99.9</b>	<b>1.440</b>	<b>99.7</b>	<b>3.729</b>	<b>99.2</b>	<b>4.004</b>	<b>93.1</b>	<b>1.277</b>
Non-extractable (PES) *	0.1	0.001	0.3	0.011	0.8	0.031	6.9	0.094
<b>Accountability</b>	<b>100.0</b>	<b>1.441</b>	<b>100.0</b>	<b>3.740</b>	<b>100.0</b>	<b>4.035</b>	<b>100.0</b>	<b>1.371</b>

Turnip and Swiss chard, 3 <sup>rd</sup> rotation	Turnip leaves		Turnip roots		Swiss chard		Swiss chard	
	mature		mature		intermediate		mature	
	%TRR	mg/kg <sup>#</sup>	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
TFA	99.9	0.992	99.8	0.086	99.7	4.769	99.7	1.967
FOE-thiadone-	n.d.**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Total</b>	<b>99.9</b>	<b>0.992</b>	<b>99.8</b>	<b>0.086</b>	<b>99.7</b>	<b>4.769</b>	<b>99.7</b>	<b>1.967</b>
Procedural loss	-	-	-	-	0.3	0.013	0.3	0.005
<b>Total</b>	<b>99.9</b>	<b>0.992</b>	<b>99.8</b>	<b>0.086</b>	<b>100.0</b>	<b>4.782</b>	<b>100.0</b>	<b>1.972</b>
Non-	0.1	0.001	0.2	<0.001	<0.1	0.002	<0.1	0.001
<b>Accountabilit</b>	<b>100.0</b>	<b>0.993</b>	<b>100.0</b>	<b>0.087</b>	<b>100.0</b>	<b>4.784</b>	<b>100.0</b>	<b>1.973</b>

\* PES = post extraction solids

\*\* n.d. = not detected

# mg/kg means mg parent equivalents/kg

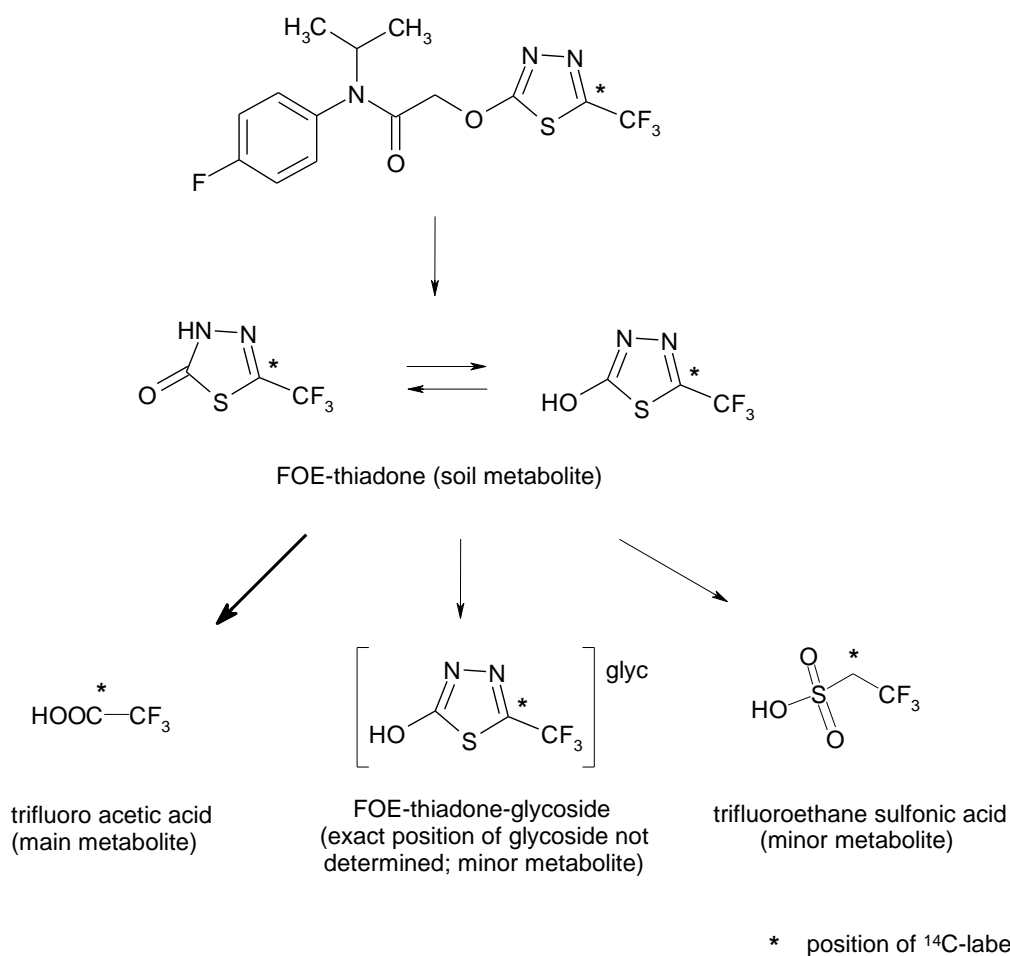
**Table 7.6.1-5: Composition of the radioactive residues in soil after application of [thiadiazole-5-<sup>14</sup>C]flufenacet at a use rate of 900 g as/ha**

Soil (0 – 15 cm)	Day 30		Day 142		Day 317	
Days after application	30		142		317	
TRR (mg equ/kg)	0.638		0.232		0.104	
	% TRR	mg/kg <sup>#</sup>	% TRR	mg/kg	% TRR	mg/kg
Flufenacet	41.9	0.267	29.0	0.069	8.6	0.009
TFA (trifluoroacetic acid)	25.4	0.162	24.6	0.059	32.5	0.034
FOE-thiadone	4.7	0.030	n.d.**	n.d.**	n.d.**	n.d.**
<b>Total identified</b>	<b>72.0</b>	<b>0.459</b>	<b>53.7</b>	<b>0.128</b>	<b>41.1</b>	<b>0.043</b>
Procedural loss	4.1	0.026	3.8	0.009	n.d.	n.d.
<b>Total extractable</b>	<b>76.1</b>	<b>0.486</b>	<b>57.5</b>	<b>0.137</b>	<b>41.1</b>	<b>0.043</b>
Non-extractable (PES) *	23.9	0.152	42.5	0.102	58.9	0.061
<b>Accountability</b>	<b>100.0</b>	<b>0.638</b>	<b>100.0</b>	<b>0.239</b>	<b>100.0</b>	<b>0.104</b>

\* PES = post extraction solids

\*\* n.d. = not detected

# mg/kg means mg parent equivalents/kg

**Figure 7.6.1- 1: Metabolic pathway of [thiadiazole-5-<sup>14</sup>C]flufenacet in rotated crops after application to bare soil at a use rate of approximately 900 g as/ha**

### SUMMARY OF THE LEVELS OF THE MAJOR METABOLITE TRIFLUOROACETATE (TFA) IN PRIMARY AND ROTATED CROPS FOLLOWING APPLICATION OF [THIADIAZOLE-5-<sup>14</sup>C]FLUFENACET

TFA (M45) has frequently been found as the main metabolite in primary and rotated crops following application of flufenacet radiolabelled in the [thiadiazole-5-<sup>14</sup>C]-position that enables the detection of TFA via radioassaying. Dietary risk assessment for TFA using a compound specific toxicological endpoint is presented in the Vol.1. In the corresponding toxicity studies TFA-Na is the relevant compound which has been dosed. Therefore, the respective residue levels of TFA from the different plant metabolism studies described above are compiled in the Table 7.6.1-6. In this table TFA residues are still given as parent equivalents. Transformation of these TFA residues to the sodium salt TFA-Na *via* the molar ratio of TFA-Na (136.01 g/mol)/flufenacet (363.34 g/mol) = 0.3743 results in Table 7.6.1-7.

**Table 7.6.1-6: Summary of TFA residues in primary and rotated crops resulting from of [thiadiazole-5-<sup>14</sup>C]flufenacet (given in flufenacet equivalents)**

3- Chlorfenacet (given in flufenacet equivalents)						
Crop	Appl. Type	Actual Appl. Rate [g as/ha]	Commodity	TFA Residue		
				[mg flufenacet equivalents/kg]		
Primary Crops						
Potato	Pre-emerg.	630	Tuber	0.801		
			Foliage	36.450		
Wheat	Post-emerg.	270	Grain	0.698		
			Straw	1.836		
			Hay	1.697		
			Forage	-		
Rotated Crops				PBI 30 days <sup>#</sup>	PBI 142 days	PBI 317 days
Wheat	Pre-plant.	903	Grain	2.899	7.624	1.277
			Straw	4.054	9.205	4.004
			Hay	3.404	8.198	3.729
			Forage	1.469	2.302	1.440
Turnip	Pre-plant.	903	Root	0.503	0.192	0.086
			Leaves	6.432	3.534	0.992
Swiss chard	Pre-plant.	903	Leaves, mat.*	3.162	2.946	1.967
			Leaves, int*	5.625	1.951	4.769

<sup>#</sup> PBI: plant back interval, interval between application of a.s. to soil and sowing of rotated crop

\* mat.: mature

int.: intermediate growth stage (50% of final leaf mass)

**Table 7.6.1-7: Summary of TFA residues in primary and rotated crops resulting from of [thiadiazole-5-<sup>14</sup>C]flufenacet (given in equivalents of TFA-Na)**

TFA Residue (given in equivalents of TFA-Na)						
Crop	Appl. Type	Actual Appl. Rate [g as/ha]	Commodity	TFA Residue [mg TFA-Na/kg]		
Primary Crops						
Potato	Pre-emerg.	630	Tuber	0.300		
			Foliage	13.644		
Wheat	Post-emerg.	270	Grain	0.261		
			Straw	0.687		
			Hay	0.635		
			Forage	-		
Rotated Crops				PBI 30 days <sup>#</sup>	PBI 142 days	PBI 317 days
Wheat	Pre-plant.	903	Grain	1.085	2.854	0.478
			Straw	1.518	3.446	1.499
			Hay	1.274	3.069	1.396
			Forage	0.550	0.862	0.539
Turnip	Pre-plant.	903	Root	0.188	0.072	0.032
			Leaves	2.408	1.323	0.371
Swiss chard	Pre-plant.	903	Leaves, mat.*	1.184	1.103	0.736
			Leaves, int*	2.106	0.730	1.785

<sup>#</sup> PBI: plant back interval, interval between application of a.s. to soil and sowing of rotated crop

\* mat.: mature

int.: intermediate growth stage (50% of final leaf mass)

In the context of TFA findings in primary and rotated crops following application of flufenacet it is kindly recommended to refer to the previous note in this dossier (chapter 7.2.1) with an explanation that TFA is formed as trifluoroacetate salt, but denoted as trifluoroacetic acid. This note is provided under the title: “Remark about formation of trifluoroacetate TFA under environmental and physiological conditions” (see B 7.2.1.3).

#### **B.7.6.2. Magnitude of residues in rotational crops**

According to the evaluation in the Monograph and by EFSA, in principle, no field rotational crop trials with flufenacet are deemed necessary to support the representative uses of flufenacet in cereals. However, field rotational crop studies were conducted at four different locations in northern Europe (northern France, Germany and the United Kingdom) on request of UK CRD to investigate the residues in treated winter cereals which are sown after the preceding crop potatoes which also received an application of a flufenacet containing product within the same calendar year. The potato crop can be considered as a representative for any possible spring crop that might be grown as a preceding crop to winter cereals. The highest registered application rates for any spring crop is 600 g as/ha.

This study has already been evaluated by UK CRD in support of flufenacet containing products to be used in cereals.

<b>Report:</b>	<b>KHIA 6.6.2/04, Melrose, I.; Erler, S.; 2008; M-306269-01</b>
Title:	Determination of the residues of FOE 5043 in/on the rotational crops cereals after spraying of Artist (41.5 WG) and Liberator (500 SC) in the field in the United Kingdom, Germany and Northern France
Document No: Study no.	M-306269-01-1 Study No. RA-2020/06 dated 2008-08-22
Guidelines:	EU-Ref: Council Directive 91/414/EEC of July 15, 1991, EC guidance working document 7029/VI/95 rev. 5 (1997-07-22) EC guidance document on rotational crop studies 7524/VI/95 rev.2 (1997-07-22)
GLP	Yes; Deviations: none

The purpose of this study was to determine the magnitude of flufenacet residues in cereals (winter wheat and winter barley) grown as rotational crops following the preceding crop potato. Potatoes and cereals were both treated with one spray application with a flufenacet containing product within the same calendar year. The study objective was to investigate whether treatment of the preceding crop with a flufenacet containing product has an impact on the residue levels determined in cereals grown as the following crop. The application rates for flufenacet correspond to the maximum registered rates for a spring crop (potatoes, maize) and cereals. The trials were performed in northern Europe (the United Kingdom, Germany and Northern France).

#### Material and methods

This study comprises four supervised residue trials with potatoes followed by cereals (2 trials on barley and wheat, each). All plots received the application of 'Flufenacet + Metribuzin 41.5 WG' to potato plants pre emergence with an application rate of 2.5 kg/ha of test item, corresponding to 600 g flufenacet /ha (and 440 g metribuzin/ha). The water rate was 300 L/ha. After harvesting potatoes, the aerial parts of the plants were incorporated into soil in order not to remove potential residues from the plot. Cereals were sown 133 - 158 days after application on potatoes. The application of 'Flufenacet + Diflufenican 500 SC' on cereals (wheat or barley) was performed between growth stages BBCH 12-22 but not later than November. The application rate was 0.6 L/ha of test item, corresponding to 240 g flufenacet /ha (and 60 g diflufenican /ha). The water rate was also 300 L/ha.

For residue analysis, samples were taken from the treated and the control plots. Only the rotational crops (cereals) were sampled for analysis and the samples were analysed only for flufenacet. Samples were collected at growth stage BBCH 30 (green material) and at harvest (BBCH 89, grain and straw).

The residues of flufenacet in/on the collected samples were determined according to the method 00346 which yields the combined level of the parent compound and all its metabolites containing the *N*-fluorophenyl-*N*-isopropyl functional group. Residues are expressed as parent flufenacet. For grain, supplement E004 (Rzepka, S.; 2006; M-277805-01 ) was applied which provides a lower LOQ for grain than the basic method. The method was modified for the clean-up of grain samples since SPE clean-up was not necessary.

The Limit of Quantification (LOQ) was 0.01 mg/kg for grain, 0.05 mg/kg for green material and 0.1 mg/kg for straw.

#### **Findings**

Recovery rates were determined prior to analysing the samples in order to validate the method, and concurrently with the sample analysis in order to check the accuracy of the residue analysis. Fortification was performed by spiking control samples with one of the following compounds or a mixture thereof: parent flufenacet, flufenacet oxalate, flufenacet sulfonic acid, flufenacet thioglycolate sulfoxide. The recovery-rates and corresponding relative standard deviations (RSD) were satisfactory, as shown in Table 7.6.2-1 for pre-validation recoveries and Table 7.6.2-2 for concurrent recoveries.

Before the analyses, samples were stored deep frozen for a maximum storage period of 12 months (371 days). The storage period is covered by the storage stability studies conducted with flufenacet.

No flufenacet residues were found in any of the untreated samples. Table 7.6.2-3 compiles the residue levels found in samples of treated cereals sown after a normal re-planting interval following potatoes which were also treated with a flufenacet containing product. The total residue of flufenacet was found to be less than the limit of quantification in green material (< 0.05 mg/kg), grain (< 0.01 mg/kg) and straw (< 0.1 mg/kg) in all treated samples.

**Table 7.6.2-1: Pre validation data for flufenacet and its metabolites on wheat grain**

Analyte	Fortification level [mg/kg]	Single Values [%]	Mean Value [%]	RSD [%]	LOQ [mg/kg]
Flufenacet (FOE 5043)	0.01	107; 102; 99; 90; 70	94	16	0.01
FOE 5043 Oxalate Hydrate	0.01	70; 90; 78; 61	75	16	0.01
FOE 5043 Sulfonic Acid Sodium Salt	0.01	71; 67; 64; 74	69	6	0.01
FOE 5043 Thioglycolate Sulfoxide	0.01	70; 78; 71; 74	73	5	0.01

Residues were determined as FOE 5043 trifluoroacetamide and expressed as flufenacet (FOE 5043) equivalents

**Table 7.6.2-2. Recovery data for flufenacet**

The LOQ is marked in bold

Study Trial No. Plot No. GLP Year	Crop	Portion analysed	a.s./metabolite	n	Fortification level (mg/kg)	Recovery (%)				
						Individual recoveries	Min	Max	Mean	RSD
RA-2020/06 R 2006 0420/3 0420-06/01  R 2006 0418/1 0418-06/01  R 2006 0003/8 0003-06/01  R 2006 0046/1 0046-06/01  GLP: yes 2006	Barley, winter	green material	Total residue flufenacet	3	<b>0.050</b>	107; 93; 82	82	107	94	13.3
				3	overall mg/kg		82	107	94	13.3
	Wheat, winter (R1)	straw	Total residue flufenacet	2	<b>0.10</b>	113; 113	113	113	113	
				2	1.00	101; 87	87	101	94	
				4	overall mg/kg		87	113	104	12.0
		grain	Total residue flufenacet	2	<b>0.010</b>	87; 91	87	91	89	
				2	0.40	84; 81	81	84	83	
				4	overall mg/kg		81	91	86	5.0

Residues were determined as FOE 5043 trifluoroacetamide and expressed as flufenacet (FOE 5043) equivalents

FOE 5043 Mix : ¼ of FOE 5043, ¼ FOE 5043 Oxalate Hydrate, ¼ of FOE 5043 Sulfonic Acid Sodium Salt, ¼ of FOE 5043 Thioglycolate Sulfoxide.

**Table 7.6.2-3: Residues of flufenacet in wheat and barley after post-emergence application of 240 g flufenacet/ha succeeding potatoes (treated with 600 g flufenacet/ha)**

Study Trial No. Trial SubID GLP Year	Crop Variety	Country	Application					Residues		
			FL	No	kg/ha (a.s.) FFA	kg/hL (a.s.) FFA	GS	Portion analysed	DALT (days)	total residue flufenacet* (mg/kg)
RA-2020/06 R 2006 0418 1 0418-06 GLP yes 2006	Potato Cilena	Germany D-49377 Vechta-Langenförden Europe, North	41.5 WG	1	0.6	0.2	00	--	--	--
	Barley, winter Franziska		500 SC	1	0.24	0.08	13	green material grain straw	164 255 255	<0.05 <0.01 <0.1
RA-2020/06 R 2006 0420 3 0420-06 GLP yes 2006	Potato Pomme Fine	France F-80700 Champien Europe, North	41.5 WG	1	0.6	0.2	00	--	--	--
	Barley, winter Colibri		500 SC	1	0.24	0.08	12	green material grain straw	97 218 218	<0.05 <0.01 <0.1
RA-2020/06 R 2006 0003 8 0003-06 GLP yes 2006	Potato Maris Peer	United Kingdom GB-SG8 85S Great Chishill Europe, North	41.5 WG	1	0.6	0.2	00	--	--	--
	Wheat, winter Consort		500 SC	1	0.24	0.08	13	green material grain straw	179 294 294	<0.05 <0.01 <0.10
RA-2020/06 R 2006 0046 1 0046-06 GLP yes 2006	Potato Cilena	Germany D-51799 Burscheid Europe, North	41.5 WG	1	0.6	0.2	00	--	--	--
	Wheat, winter Limes		500 SC	1	0.24	0.08	21	green material grain straw	147 277 277	<0.05 <0.01 <0.1

\*Residues for total residue flufenacet determined as FOE 5043 Trifluoro acetamide and calculated as flufenacet  
 DALT : Days after last treatment; FFA Flufenacet; GS: Growth stage

## Conclusion

Four field residue trials were conducted in northern Europe (United Kingdom, Germany and France) in order to determine the magnitude of flufenacet derived residues in/on cereals (winter wheat and winter barley) grown as succeeding crops following the preceding crop potatoes. Potatoes and cereals were both treated with one spray application of a flufenacet containing product (at the maximum rates of 600 g as/ha for potatoes and 240 g as/ha for cereals). No residues were apparent in green material of cereals collected at growth stage BBCH 29 – 30 or grain and straw sampled at harvest (BBCH 89). The findings show that treatment of the preceding crop with a flufenacet containing product at the maximum field rate does not impact residue levels in/on cereals grown as succeeding crops. No uptake from the soil into the following crop has been observed. This scenario reflects a worst case rotation with regard to potential uptake from soil. Shorter plant back intervals (e.g. 30 days) were not investigated since the time for sowing spring cereals has already passed in case of failure of other spring crops (i.e. potatoes, maize) that may have received a treatment with a flufenacet. The absence of residues in cereals when sown as following crop is considered to be representative for all other rotational crop situations where the preceding crop is treated with application rates up to 600 g as/ha.

Flufenacet residues were found to be less than the limit of quantification of 0.01 mg/kg in grain, 0.05 mg/kg in green material and 0.1 mg/kg in straw.

### B.7.7. OTHER STUDIES

#### B.7.7.1. Effect on the residue level in pollen and bee products

The objective of such studies would be to determine the residues in pollen and bee products for human consumption resulting from residues taken up by honeybees from crops at blossom.

No final test guideline is currently available which provides an agreed test methodology. Therefore it is not appropriate to address this issue until such guidance is available (cf. also. ‘Guidance Document for Applicants on Preparing Dossiers for the Approval of a Chemical New Active Substance and For the Renewal of Approval of a Chemical Active Substance According to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013’).

Flufenacet is applied to cereals pre-emergence or at early stages of plant development during leaf development or tillering before blossom. Furthermore, residues are very low in all plant commodities investigated. Also, cereals are typically no feeding item for bees. Therefore, any studies to investigate residues in pollen and bee products as a result of flufenacet uses in cereals are not considered necessary.

#### B.7.7.2. The toxicological profile and exposure assessment of metabolites

The toxicological profile and exposure assessment of metabolites in food of plant origin is addressed in an applicant’s position paper cited below, also provided in section 5 (toxicological and metabolism studies).

<b>Report:</b>	<b>KCA 6.10/01</b> Buerkle, L., Hartmann, K., Weile, M.; 2014; M-476535-01_
Title:	Flufenacet - Toxicological profile and exposure assessment of the plant metabolites
Document No: Study no.	M-476535-01-1
Guidelines:	Not applicable (position paper)
GLP	Not applicable (position paper)

Flufenacet is both rapidly and extensively metabolised, such that even at early sampling dates no parent compound is detected in plant commodities. A detailed comparison of plant and rat metabolism reveals that several plant metabolites were not detected as systemic metabolites in rat ADME studies.

For flufenacet (including its metabolites) a comprehensive toxicological database exists which was already evaluated during the peer review under Directive 91/414/EEC to a great extent. In the context of the application for renewal of approval of the active substance flufenacet according to Regulation (EC) 1107/2009 the toxicological data base has even been extended by several new toxicological studies.

The toxicological characterization of several plant metabolites containing either the fluorophenyl isopropyl amine moiety or the thiadone moiety shows that an additional toxicological impact from these compounds is not expected.

Due to structural similarity considerations, the absence of a genotoxicity potential, some further toxicity studies and supplementary information from metabolism studies with FOE sulfonic acid (M02), FOE oxalate (M01), thiadone-*N*-glucoside (M25) and trifluoroacetate (M45) the plant metabolites containing the fluorophenyl isopropyl amine moiety as well as the metabolites derived from the thiadone moiety are not expected to exert higher toxicity or additional hazards beyond those identified for flufenacet.

The metabolites derived from the fluorophenyl isopropyl acetamide moiety are included in the established residue definition by means of a common moiety approach. For two metabolites (FOE sulfonic acid and FOE oxalate) supplementary information is available from metabolism studies in rats and ruminants and feeding studies with FOE oxalate in cattle and poultry. These studies show their metabolic stability and low bio-availability. Thus, it seems to be justified to use the toxicological endpoints of the parent compound for the risk assessments.

From the long-term and short-term consumer exposure calculations for the metabolites containing the fluorophenyl isopropyl amine moiety it can be concluded that possible intakes do not present a consumer health concern.

The risk assessments performed for FOE oxalate and FOE sulfonic acid which may contribute through possible occurrence in food of plant origin and in drinking water demonstrate that the toxicological reference values are not exhausted also when combining both sources of exposure.

Thiadone-*N*-glucoside (M25) is a plant metabolite originating from the thiadiazole part of the parent compound. Thiadone-*N*-glucoside is a polar metabolite which is excreted in rats without undergoing further metabolism or cleavage of glucose as evident from a supplementary goat metabolism study. The experiment showed low bio-availability and the metabolic stability of thiadone-*N*-glucoside. However, free thiadone may be formed in ruminants after ingesting feeding items containing thiadone-*N*-glucoside. Taking into account the findings from the supplementary goat metabolism study with overdosed thiadone-*N*-glucoside the human dietary burden of free thiadone anticipated in food of animal origin is considered to be minimal.

Considering that thiadone is a major rat metabolite, the toxicological properties can be considered to be co-tested with the parent compound flufenacet.

Trifluoroacetate (TFA, M45) is a major plant metabolite observed in primary and rotational crops. Based on the toxicological information it is deemed justified to conduct the dietary risk assessment using a specific toxicological endpoint for this metabolite. It can be concluded that exposure arising from uses of flufenacet does not result in a consumer health concern, also when taking into account possible contributions from drinking water as an additional source. The TFA concentrations used in the risk assessment are considered to be sufficiently conservative and, in practice, the actual intake is likely to be much lower than the calculated values. When applying several worst case assumptions the calculations indicate that the intended use of flufenacet containing products does not pose a risk to consumers as a result of exposure to TFA.

**Conclusion:**

*Thiadone and thiadiazole metabolites (especially TFA) are not qualitatively and quantitatively relevant. Consumer risk assessment for TFA is included in Vol.1.*

## **B.7.8. REFERENCES RELIED ON**

### **Scientific peer-reviewed open literature**

For AIR 3 compounds such as flufenacet a literature review is mandatory for the Annex I Renewal.

A literature review for flufenacet and its' metabolites was carried out by the applicant according to the requirements of the Regulation (EU) No 844/2012, which itself refers to Article 8(5) of Regulation (EC) No 1107/2009. The full description of this review is provided in Document MCA, Section 9.

Time window of the literature search was January 1<sup>st</sup>, 2000 – November 13<sup>th</sup>, 2013.

The applicant has used a broad collection of relevant databases for the literature search, namely: Agricola, Biosis, Chemical Abstracts, Derwent Drug File DRUGU), EMBASE, Esbiobase, IPA, Medline, Pascal, PQSciTech, Registry, Scisearch, Toxcenter, Ulidat and FSTA. STN, a scientific information platform hosted by CAS, itself a division of the American Chemical Society, was selected as the preferred provider.

The input parameters for the database search on flufenacet and its metabolites included IUPAC name, CAS number, common name(s), code and abbreviation, molecular structure, molecular formula, other names/codes.

A total of 3489 references were identified and evaluated for potential relevance (369 for flufenacet and 3120 for its metabolites). Abstracts of all references have been downloaded and evaluated.

The first rapid assessment step focused on the potential relevance of the studies, based on the abstracts. 3336 publications (278 for flufenacet and 3058 for metabolites) were excluded at this step.

Those publications, which have passed the rapid assessment (153 in total, 91 for flufenacet, 62 for metabolites), have been evaluated in details based on their full text versions. The number of publications excluded from further consideration after detailed assessment of relevance was 144 (89 for flufenacet, 55 for metabolites). Finally, 7 publications were regarded as potentially relevant (none of them concerned residue section). However, no reference was identified as relevant in the context of side-effects on health, the environment and non-target species, which does influence the risk assessment (as defined in the EFSA Guidance Document, EFSA Journal 2011;9(2):2092).

RMS considers that the review is acceptable in terms of databases searched and the search criteria applied. The search did not reveal any references of relevance to the residues section. As a consequence the risk assessments in the supplementary dossier remain fully valid after consideration of all search results.

In section B.7, reference has been made to EFSA's Reasoned Opinion on the review of existing maximum residue levels (MRLs) for flufenacet according to Article 12 of Regulation (EC) No 396/2005 (EFSA Journal 2012;10(4):2689).

### **List of studies relied upon**

The reference list below includes the newly submitted data relied on as well as the original submitted tests and studies that are still considered relevant and therefore used to support the renewal of the a.s. approval. Old references are marked with grey font, while for the new ones, black font have been applied.

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.1 /01	Bosnak, L. L.	1995	The storage stability of FOE 5043 and metabolites in corn, soybean, and turpin raw agricultural commodities - Addendum 1 - The storage stability of FOE 5043 and metabolites in corn, soybean, and turnip raw agricultural commodities- 20-month and 28-month data Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 106971, Edition Number: M-002426-01-1 EPA MRID No.: 44228701 Date: 1995-08-16 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.1 /02	Stuke, S.; Ballmann, C.	2012	Amendment no. 1 to report no: P642100741 - Storage stability of flufenacet and metabolites in/on orange fruit and dry bean seeds for 24 months Bayer CropScience, Report No.: MR-10/006, Edition Number: M-439517-02-1 Date: 2012-10-08 <b>...Amended: 2013-11-05</b> GLP/GEP: yes, unpublished	N	Y	Completion of data package on storage stability (additional matrix groups)	Bayer CropScience
KCA 6.1 /03	Klimmek, S.; Gizler, A.	2013	Amendment No. 1 to Final Report - 7 days freezer storage stability study of flufenacet (FOE5043), and its metabolites in tomato and wheat grain Eurofins Agrosience Services Chem GmbH, Hamburg, Germany Bayer CropScience, Report No.: S13-02753, Edition Number: M-467724-02-1 Date: 2013-10-08 <b>...Amended: 2013-11-19</b> GLP/GEP: yes, unpublished	N	Y	Short-term storage stability study to address temperature deviations during shipment of field samples	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.1 /04	Bosnak, L. L.	1997	The storage stability of FOE 5043 and metabolites in wheat forage, grain, and straw Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 107137, Edition Number: M-002424-01-1 EPA MRID No.: 45012402 Date: 1997-04-22 GLP/GEP: yes, unpublished	N	Y	Data generated for USA	Bayer CropScience
KCA 6.2.1 /01	Baird, J. H.	1994	Metabolism of [fluorophenyl-UL-14C] FOE 5043 in corn Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR105027, Edition Number: M0022270-01-1 Date: 1994-12-19 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.2.1 /02	Krolski, M. E.; Bosnak, L. L.	1995	The metabolism of FOE 5043 in soybeans Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR105187, Edition Number: M-002278-01-1 Date: 1995-03-07 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KCA 6.2.1 /03	Krolski, M. E.; Bosnak, L. L.	1995	The metabolism of [Fluorophenyl-UL-14C] FOE 5043 in cotton Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: MR106666, Edition Number: M-002277-01-1 Date: 1995-12-01 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.1 /04	Koester, J.; Brauner, A.	1995	Degradation of [Fluorophenyl-UL-14C]FOE 5043 and [Thiadiazole-2-14C]FOE 5043 by heterotrophic plant cell suspension cultures (supplemental study in support of biodegradation in plants) Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: PF4049, Edition Number: M-002366-01-1 Date: 1995-04-12 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.2.1 /05	Krolski, M. E.; Bosnak, L. L.	1998	The metabolism of [fluorophenyl-UL-14C] FOE 5043 in corn after postemergent foliar spray application Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 108497, Edition Number: M-005755-01-1 Date: 1998-09-23 GLP/GEP: yes, unpublished	N	Y	Completion of data package, post-emergence use on maize	Bayer CropScience
KCA 6.2.1 /06	Bongartz, R.	2012	Metabolism of [thiadiazole-5-14C]flufenacet in potatoes - Final report Bayer CropScience, Report No.: EnSa-12-0537, Edition Number: M-441506-02-1 Date: 2012-11-07 <b>...Amended: 2012-12-10</b> GLP/GEP: yes, unpublished	N	Y	Completion of data package, additional crop, new label	Bayer CropScience
KCA 6.2.1 /07	Bongartz, R.; Miebach, D.	2013	Metabolism of [thiadiazole-5-14C]flufenacet in wheat Bayer CropScience, Report No.: EnSa-12-0536, Edition Number: M-444475-01-1 Date: 2013-01-07 GLP/GEP: yes, unpublished	N	Y	Completion of data package, new label	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.2 /01	[REDACTED]	1995	Metabolism of [Fluorophenyl-UL-14C] FOE 5043 in laying hens Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR103946, Edition Number: M-002251-01-1 Date: 1995-03-24 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KCA 6.2.2 /02	[REDACTED]	1995	Metabolism of [Thiadiazole-2-14C] FOE 5043 in laying hens Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR106785, Edition Number: M-002253-01-1 Date: 1995-03-20 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KCA 6.2.2 /03	[REDACTED]	1995	Metabolism of [phenyl-UL-14C] FOE oxalate in laying hens Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR106787, Edition Number: M-004474-01-1 Date: 1995-04-11 GLP/GEP: yes, unpublished ...also filed: KCA 5.1 /01	Y	N		Bayer CropScience
KCA 6.2.2 /04	[REDACTED]	2013	[1-14C]Trifluoroacetic acid: Metabolism in the laying hen Bayer CropScience, Report No.: EnSa-12-0648, Edition Number: M-463376-01-1 Date: 2013-09-02 GLP/GEP: yes, unpublished	Y	Y	Data on major metabolite; new information: animal metabolism of TFA metabolite in laying hen	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.3 /01	██████████ ██████████	1995	Metabolism of [Fluorophenyl-UL-14C] FOE 5043 in a lactating goat Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR105184, Edition Number: M-002250-01-1 Date: 1995-03-03 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KCA 6.2.3 /02	██████████ ██████████ ██████████	1995	Metabolism of [Thiadiazole-2-14C] FOE 5043 in a lactating goat Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: MR106784, Edition Number: M-002248-01-1 Date: 1995-07-11 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience
KCA 6.2.3 /03	██████████ ██████████ ██████████ ██████████	1995	Metabolism of [phenyl-UL-14C] FOE oxalate in a lactating goat Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: MR106786, Edition Number: M-004478-01-1 Date: 1995-03-31 GLP/GEP: yes, unpublished ...also filed: KCA 5.1 /02	Y	N		Bayer CropScience
KCA 6.2.3 /04	██████████ ██████████	2002	The metabolism of FOE 5043 thiadone N-glycoside in a lactating goat Bayer Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 110998, Edition Number: M-079251-01-1 Date: 2002-05-21 GLP/GEP: yes, unpublished	Y	Y	Study conducted on request of US EPA. No identification of residues in edible tissues and milk.	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.2.3 /05	██████ ██████ ██████	2013	[1-14C]Trifluoroacetic acid - Metabolism in the lactating goat Bayer CropScience, Report No.: EnSa-12-0628, Edition Number: M-444459-01-1 Date: 2013-01-08 GLP/GEP: yes, unpublished	Y	Y	new information: animal metabolism of TFA metabolite in lactating goat; Transfer study on major metabolite	Bayer CropScience
KCA 6.2.5 /01	██████ ██████	1994	Identification of radioactive residues of Phenyl-(14C) FOE 5043 in bluegill sunfish (Lepomis macrochirus) Bayer Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 106577, Edition Number: M-003804-01-1 EPA MRID No.: 43441139 Date: 1994-07-13 GLP/GEP: yes, unpublished ...also filed: <b>KCA 8.2.1 /04</b>	Y	N	New data requirement (fish metabolism)	Bayer CropScience
KCA 6.2.5 /02	██████	1994	Uptake, depuration and bioconcentration of 14C-FOE 5043 technical by bluegill (Lepomis macrochirus) under flow-through conditions Bayer Corporation, Kansas City, MO, USA Bayer CropScience, Report No.: 106760, Edition Number: M-003803-01-1 EPA MRID No.: 43441127 Date: 1994-07-08 GLP/GEP: yes, unpublished ...also filed: <b>KCA 8.2.2.3 /01</b>	Y	N	New data requirement (fish metabolism)	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /01	Seym, M.	1996	Determination of residues of FOE 5043 60 WG in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany and France Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2008/94, Report includes Trial Nos.: 400351 400378 400386 400394 401528 401544 Edition Number: M-002280-01-2 Date: 1996-03-25 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /02	Jersch-Schmitz, S.; Seym, M.	1995	Determination of residues of FOE 5043 60 WG in/on winter wheat and winter barley following early post-emergence spray application in Germany, France and the Netherlands Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2054/93, Report includes Trial Nos.: 300489 301655 301663 301671 302554 302562 305030 305049 305057 305065 305138 305146  Edition Number: M-002284-01-2 Date: 1995-10-10 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /03	Seym, M.	1996	Determination of residues of FOE 5043 & Diflufenican 60 WG in/on winter barley, winter rye and winter wheat following early post-emergence spray application in Germany Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2010/94, Report includes Trial Nos.: 400440 400459 400467 400475 Edition Number: M-004451-01-2 Date: 1996-03-25 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience
KCA 6.3.1 /04	Seym, M.; Deissler, A.	1999	Determination of residues of FOE 5043 & Diflufenican 70 WG in/on winter barley and winter wheat in the field in France Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2153/97, Report includes Trial Nos.: 702587 707317 707325 Edition Number: M-012486-02-1 Date: 1999-02-10 <b>...Amended: 1999-07-29</b> GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /05	Neigl, A.	2000	Determination of residues of FOE 5043 on winter wheat after spray application of FOE 5043 & diflufenican 70 WG in the field in France Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2185/98, Report includes Trial Nos.: 817260 817279 Edition Number: M-033163-01-1 Date: 2000-05-12 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience
KCA 6.3.1 /06	Hoffmann, M.	2002	Determination of residues of FOE 5043 in/on wheat and barley following spray application of FOE 5043 & Diflufenican (600 SC) to winter wheat and winter barley in the field in Northern and Southern France, Germany and Spain Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2144/00, Report includes Trial Nos.: R 2000 0566/0 R 2000 0567/9 R 2000 0568/7 R 2000 0570/9 Edition Number: M-058156-01-1 Date: 2002-04-12 GLP/GEP: yes, unpublished	N	Y	Supplementary data (N-EU) and new (S-EU) on supported crop/GAP	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /07	Billian, P.; Krusell, L.	2010	Determination of the residues of diflufenican and flufenacet in/on winter barley after spraying of Flufenacet & Diflufenican SC 600 in the field in France (South) Bayer CropScience, Report No.: 09-2048, Report includes Trial Nos.: 09-2048-01 09-2048-02 09-2048-03 Edition Number: M-361495-01-1 Date: 2010-01-12 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience
KCA 6.3.1 /08	Billian, P.; Reineke, A.; Krusell, L.	2010	Determination of the residues of diflufenican and flufenacet in/on winter wheat after spraying of Flufenacet & Diflufenican SC 600 in the field in France (south) Bayer CropScience, Report No.: 09-2052, Report includes Trial Nos.: 09-2052-01 09-2052-02 09-2052-03 09-2052-04 Edition Number: M-363200-02-1 Date: 2010-02-05 <b>...Amended: 2010-08-05</b> GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /09	Noss, G.; Diehl, P.	2013	Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter wheat after spray application of DFF & FFA & FLT SC 360 in Germany, the Netherlands, southern France and Spain - Diflufenican + flufenacet + flurtamone SC 360 (120 + 120 + 120 g / L) Bayer CropScience, Report No.: 11-2095, Report includes Trial Nos.: 11-2095-01 11-2095-02 11-2095-03 11-2095-04 Edition Number: M-459755-01-1 Date: 2013-07-10 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience
KCA 6.3.1 /10	Stuke, S.; Ballmann, C.	2013	Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after Spraying of DFF & FFA & FLT SC 360 in the field in Germany, Belgium and the Netherlands - DFF+FFA+FLT SC 120+120+120 Bayer CropScience, Report No.: 12-2001, Report includes Trial Nos.: 12-2001-01 12-2001-02 12-2001-03 12-2001-04 Edition Number: M-459795-01-1 Date: 2013-07-09 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /11	Stuke, S.; Teubner, L.; Ballmann, C.	2013	Determination of the residues of flufenacet and flurtamone in/on winter barley and winter wheat after spray application of DFF & FFA & FLT SC 360 in Southern France, Italy, Spain and Portugal - DFF+FFA+FLT SC 120+120+120 G U-EU Bayer CropScience, Report No.: 12-2002, Report includes Trial Nos.: 12-2002-01 12-2002-02 12-2002-03 12-2002-04 Edition Number: M-459799-01-1 Date: 2013-07-09 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience
KCA 6.3.1 /12	Noss, G.; van Berkum, S.	2013	Determination of the residues of diflufenican, flufenacet and flurtamone in/on winter barley after spray application of DFF & FFA & FLT SC 360 in Germany, the United Kingdom, southern France and Italy - Diflufenican + flufenacet + flurtamone SC 360 (120 + 120 + 120 g / L) Bayer CropScience, Report No.: 11-2094, Report includes Trial Nos.: 11-2094-01 11-2094-02 11-2094-03 11-2094-04 Edition Number: M-460003-01-1 Date: 2013-07-11 GLP/GEP: yes, unpublished	N	Y	Supplementary data on supported crop/product/GAP	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.1 /13	Weile, M.	2014	Tier 1 summary of the residue data and processing studies for flufenacet and supplementary residue data supporting the representative product flufenacet + diflufenican SC 600 Bayer CropScience, Report No.: M-478066-01-1, Edition Number: M-478066-01-1 Date: 2014-02-10 GLP/GEP: n.a., unpublished	N	N		Bayer CropScience
KCA 6.3.2 /01	Seym, M.	1995	Determination of residues of FOE 5043 60 WG in corn following preemergence spray application in Germany and France Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2009/94, Report includes Trial Nos.: 400408 400416 400424 400432 401560 401579 Edition Number: M-002297-02-1 Date: 1995-08-07 ...Amended: 1995-10-20 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.2 /02	Seym, M.	1995	Determination of residues of FOE 5043 60 WG in corn following preemergenc spray application in France Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2052/93, Report includes Trial Nos.: 302406 302414 302422 302430 Edition Number: M-002304-02-2 Date: 1995-07-20 <b>...Amended: 1995-10-20</b> GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.3.2 /03	Seym, M.	1995	Determination of residues of FOE 5043 60 WG on corn in France, Italy and Greece Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2067/94, Report includes Trial Nos.: 401587 402761 402788 402796 404306 404969 Edition Number: M-002293-01-2 Date: 1995-11-20 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.3 /01	Heinemann, O.; Seym, M.	1995	Determination of residues of FOE 5043 60 WG on sunflowers following pre-emergence spray application in France and Italy Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2040/94, Report includes Trial Nos.: 401668 401676 401684 401692 Edition Number: M-002381-01-2 Date: 1995-10-16 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.3.3 /02	Heinemann, O.; Seym, M.	1995	Determination of residues of FOE 5043 60 WG on sunflowers following pre-emergence or early post- emergence spray application in France Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2053/93, Report includes Trial Nos.: 302643 302651 302678 302686 Edition Number: M-002376-01-2 Date: 1995-10-16 GLP/GEP: yes, unpublished	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.3.4 /01	Heinemann, O.; Seym, M.	1995	Determination of residues of FOE 5043 60 WG on soya following pre-emergence spray application in Italy Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2068/94, Report includes Trial Nos.: 402818 402826 402834 402842 Edition Number: M-002387-01-2 Date: 1995-10-16 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.3.4 /02	Seym, M.	1997	Determination of residues of FOE 5043 60 WG following pre-emergent spray application on soybean in Italy Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: RA-2079/95, Report includes Trial Nos.: 500526 505676 505714 505722 Edition Number: M-002384-01-2 Date: 1997-04-28 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.4.2 /01	██████	1995	FOE Oxalate - a 29-day dairy cattle feeding study Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: MR106945, Edition Number: M-002268-01-1 Date: 1995-09-27 GLP/GEP: yes, unpublished	Y	N		Bayer CropScience

**Flufenacet**
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<b>Annex point / reference number</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not</b>	<b>Vertebrate study Y/N</b>	<b>Data protection claimed Y/N</b>	<b>Justification if data protection is claimed</b>	<b>Owner</b>
KCA 6.5.1 /01	Buerkle, L.W.	2011	Flufenacet - Waiving of the high temperature hydrolysis study for the determination of the nature of pesticides residues in processed commodities Bayer CropScience, Report No.: MEF-11/482, Edition Number: M-409521-01-1 Date: 2011-06-10 GLP/GEP: no, unpublished	N	Y	Waiver for GL requirement	Bayer CropScience
KCA 6.5.3 /01	Grace, T. J.	1995	FOE 5043 60 DF - Magnitude of the residue in corn processed products Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 106659, Edition Number: M-002412-01-1 Date: 1995-08-02 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.5.3 /02	Grace, T. J.	1995	FOE 5043 60 DF - Magnitude of the residue in soybean processed products Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 106668, Edition Number: M-002420-01-1 Date: 1995-08-02 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.5.3 /03	Krolski, M. E.	1997	FOE 5043 60 DF - Magnitude of the residue in wheat processed commodities and aspirated grain fractions Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 107840, Edition Number: M-002403-01-1 EPA MRID No.: 45012408 Date: 1997-12-03 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.5.3 /04	Noss, G.; Ruhl, S.; Ballmann, C.	2013	Determination of the residues of flufenacet in/on wheat and the processed fractions (white flour, white flour bran, white bread, whole meal, whole meal bread, middlings, shorts, gluten, gluten feed meal, starch) after spraying of Flufenacet WG 60 in the United Kingdom and the Netherlands Bayer CropScience, Report No.: 11-3401, Report includes Trial Nos.: 11-3401-01 11-3401-02 Edition Number: M-457286-01-1 Date: 2013-06-20 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer CropScience
KCA 6.5.3 /05	Noss, G.	2013	Determination of the residues of flufenacet in/on barley and the processed fractions from pearl barley processing and preparation of alcoholic beverages (malting, brewing, distillation) after spray application of Flufenacet WG 60 in Germany and Belgium Bayer CropScience, Report No.: 11-3400, Report includes Trial Nos.: 11-3400-01 11-3400-02 Edition Number: M-468736-02-1 Date: 2013-01-25 <b>...Amended: 2014-01-07</b> GLP/GEP: yes, unpublished	N	Y	based on guideline requirement	Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.6 /01	Krolski, M. E.	1997	FOE 5043 60 DF - Magnitude of the residue in field rotational crops Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 107703, Edition Number: M-002371-01-1 EPA MRID No.: 44253103 Date: 1997-03-27 GLP/GEP: yes, unpublished ...also filed: <b>KCA 6.6.2 /03</b>	N	N		Bayer CropScience
KCA 6.6.1 /01	Halarnkar, P. P.; Mennicke, E. J.	1995	Accumulation of [Thiadiazole-2-14C]FOE 5043 residues in confined rotational crops Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: MR106639, Edition Number: M-002368-01-1 Date: 1995-05-26 GLP/GEP: yes, unpublished	N	N		Bayer CropScience
KCA 6.6.1 /02	Lenz, M. F.; McKinney, M. K.	1994	Accumulation of [Phenyl-14C] FOE 5043 residues in confined rotational crops Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR106768, Edition Number: M-002369-01-1 Date: 1994-10-17 GLP/GEP: yes, unpublished ...also filed: <b>KCA 6.6.2 /01</b>	N	N		Bayer CropScience

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Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.6.1 /03	Bongartz, R.; Klankers, M.	2012	Metabolism of [thiadiazole-5-14C]Flufenacet in confined rotational crops Bayer CropScience, Report No.: EnSa-12-0535, Edition Number: M-443538-01-1 Date: 2012-11-29 GLP/GEP: yes, unpublished	N	Y	Completion of data package, new label	Bayer CropScience
KCA 6.6.2 /01	Lenz, M. F.; McKinney, M. K.	1994	Accumulation of [Phenyl-14C] FOE 5043 residues in confined rotational crops Miles Inc., Agriculture Division, Stilwell, KS, USA Bayer CropScience, Report No.: MR106768, Edition Number: M-002369-01-1 Date: 1994-10-17 GLP/GEP: yes, unpublished ...also filed: <b>KCA 6.6.1 /02</b>	N	N		Bayer CropScience
KCA 6.6.2 /02	Brauner, A.; Klamroth, E.	1999	FOE 5053 residues in succeeding crops Bayer CropScience, Report No.: M 10154, Edition Number: M-275224-01-1 Date: 1999-03-16 GLP/GEP: n.a., unpublished	N	N		Bayer CropScience
KCA 6.6.2 /03	Krolski, M. E.	1997	FOE 5043 60 DF - Magnitude of the residue in field rotational crops Bayer Corporation, Stilwell, KS, USA Bayer CropScience, Report No.: 107703, Edition Number: M-002371-01-1 EPA MRID No.: 44253103 Date: 1997-03-27 GLP/GEP: yes, unpublished ...also filed: <b>KCA 6.6 /01</b>	N	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.6.2 /04	Melrose, I.; Erler, S.	2004	Determination of the residues of FOE 5043 in/on the rotational crops cereals after spraying of Artist (41.5 WG) and Liberator (500 SC) in the field in the United Kingdom, Germany and Northern France Bayer CropScience S.A., Lyon, France Bayer CropScience, Report No.: RA-2020/06, Report includes Trial Nos.: R2006-0003/8=0003-06 R20060046/1=0046-06 R20060418/1=0418-06 R20060420/3=0420-06 Edition Number: M-306269-01-1 Date: 2004-07-19 GLP/GEP: yes, unpublished	N	Y	New data on rotational crops	Bayer CropScience
KCA 6.9 /01	Anon.	1993	Pruefung des Rueckstandsverhaltens - Abschaetzung der Aufnahme von Pflanzenschutzmittelrueckstaenden ueber die Nahrung Publisher:Saphir Verlag, Location:Ribbesbüttel, Journal:Biologische Bundesanstalt für Land- und Forstwirtschaft, Volume:Teil IV, Pages:1-52, Year:1993, Report No.: MO-98-000905, Edition Number: M-002038-01-1 GLP/GEP: n.a., published	N	N		

Annex point / reference number	Author(s)	Year	Title Source ( <i>where different from company</i> ) Company name, Report No., Date, GLP status ( <i>where relevant</i> ), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 6.9 /02	Anon.	1995	Details of theoretical maximum daily intake and estimated maximum daily intake calculations for pesticides evaluated by the 1994 joint FAO/WHO meeting on pesticides residues, including global/cultural diets Publisher:Joint FAO/WHO, Location:Hague, Netherlands, Journal:Conference room document 5, Year:1995, Report No.: MO-98-000907, Edition Number: M-002041-01-1 GLP/GEP: n.a., published	N	N		
KCA 6.9 /03	Anon.	1989	Guidelines for predicting dietary intake of pesticide residues Publisher:World Health Organization, Location:Geneva/Switzerland, Pages:1-24; +2, Year:1989, Report No.: MO-98-000909, Edition Number: M-002043-01-1 GLP/GEP: n.a., published	N	N		

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**Appendix 1**
**1) Residue trials (Northern Europe, Data relevant for Annex I listing (peer reviewed))**

<b>Report</b>	<b>Crop</b>	<b>Application rate Flufenacet [g as/ha]</b>	<b>Growth stage</b>	<b>Number of trials</b>
<b>RA-2008/94</b>	<b>Barley</b>	<b>240-260</b>	<b>BBCH 13-21</b>	<b>2</b>
	<b>Rye</b>	<b>240</b>	<b>BBCH 21-25</b>	<b>2</b>
	<b>Wheat</b>	<b>186-240</b>	<b>BBCH 13-21</b>	<b>2</b>
<b>RA-2054/93</b>	<b>Barley</b>	<b>240</b>	<b>BBCH 11-24</b>	<b>5</b>
	<b>Wheat</b>	<b>240</b>	<b>BBCH 11-22</b>	<b>7</b>

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2008/94 40154/4 0154-94 France, north 27150 La Broche 1994	Barley, winter Plaisant	1) 29.09.1994 2) 20.05.1995 - 01.06.1995 3) 07.07.1995	SPI	0.2604	304	0.08580	21.11.1994/0	Beginning of tillering	green material	<0.05 <0.05	120 169	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									grain	<0.05	228	
									straw	<0.1	228	(h) 0.1 mg/kg

cross reference: KCA 6.3.1 (M-002280-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/  (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2008/94 40035/1 0035-94 Germany Versuchsgut Höfchen, 51399 Burscheid 1994	Barley, winter Loreley	1) 22.09.1994 2) 29.05.1995 - 04.06.1995 3) 13.07.1995	SPI	0.2400	300	0.07980	02.11.1994/0	3 leaves unfolded	green material	<0.05 <0.05	124 201	(c) SPI: Spraying (g) 00346 (h) 0.05 mg/kg
									grain	<0.05	253	
									straw	<0.1	253	

cross reference: KCA 6.3.1 (M-002280-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2008/94 40037/8 0037-94 Germany Versuchsgut Laacherhof, D- 40789 Monheim 1994	Rye, winter Gambit	1) 10.10.1994 2) 24.05.1995 - 30.05.1995 3) 25.07.1995	SPI	0.2400	300	0.07980	21.11.1994/0	Beginning of tillering	green material   grain  straw	<0.05 <0.05   <0.05  <0.1	94 172   246  246	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg      (h) 0.1 mg/kg

cross reference: KCA 6.3.1 (M-002280-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2008/94 40038/6 0038-94 Germany Versuchsgut Höfchen, 51399 Burscheid 1994	Rye, winter Amilo	1) 29.09.1994 2) 27.05.1995 - 03.06.1995 3) 26.07.1995	SPI	0.2400	300	0.07980	21.11.1994/0	5 tillers detectable	green material      grain   straw	0.05 <0.05      <0.05  <0.1	88 172      247  247	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg        (h) 0.1 mg/kg

cross reference: KCA 6.3.1 (M-002280-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2008/94 40039/4 0039-94 Germany Versuchsgut Laacherhof, 40789 Monheim 1994	Wheat, winter Contra	1) 12.10.1994 2) 10.06.1995 - 17.06.1995 3) 25.07.1995	SPI	0.2400	300	0.07980	21.11.1994/0	Beginning of tillering	green material	0.09 <0.05	133 190	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									grain	<0.05	246	
									straw	<0.10	246	(h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002280-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2008/94 40152/8 0152-94 France, north 27700 Guiseniers 1994	Wheat, winter Soisson	1) 18.10.1994 2) 12.06.1995 - 24.06.1995 3) 20.07.1995	SPI	0.186 0	217	0.08580	02.12.1994/0	3 leaves unfolded	green material	<0.05 <0.05	111 174	(c) SPI: Spraying (g) 00346 (h) 0.05 mg/kg
									grain	<0.05	230	
									straw	<0.10	230	

cross reference: KCA 6.3.1 (M-002280-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30165/5 0165-93 Germany Versuchsgut Höfchen, 51399 Burscheid 1993	Barley, winter Loreley	1) 20.09.1993 2) 25.05.1994 - 29.05.1994 3) 11.07.1994	SPI	0.2400	300	0.07980	13.10.1993/0	2 leaves unfolded	green material	<0.05 <0.05	189 219	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									grain	<0.05	271	
									straw	<0.10	271	

cross reference: KCA 6.3.1 (M-002284-01-1)

**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30167/1 0167-93 Germany Versuchsgut Laacherhof, 40789 Monheim 1993	Barley, winter Jana	1) 28.09.1993 2) 25.05.1994 - 29.05.1994 3) 11.07.1994	SPI	0.2400	300	0.07980	20.10.1993/0	First leaf unfolded	green material	<0.05 <0.05	162 210	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									grain	<0.05	264	
									straw	<0.10	264	(h) 0.10 mg/kg

cross reference:KCA 6.3.1 (M-002284-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30255/4 0255-93 France, north 27700 Fresne l'Archeveque 1994	Barley, winter Glenan	1) 18.10.1993 2) 16.05.1994 - 31.05.1994 3) 11.07.1994	SPI	0.2400	280	0.08580	09.03.1994/0	2 tillers detectable	green material  grain  straw	0.19 <0.05  <0.05  <0.10	20 62  124  124	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg
RA-2054/93 30504/9 0504-93 Germany Versuchsgut Höfchen, 51399 Burscheid 1994	Barley, winter Marinka	1) 01.10.1993 2) 10.06.1994 - 14.06.1994	SPI	0.2400	300	0.07980	11.01.1994/0	Beginning of tillering	green material	<0.05 <0.05	111 146	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg

cross reference:KCA 6.3.1 (M-002284-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30513/8 0513-93 France, north 27630 Berthenonville 1994	Barley, winter Plaisant	1) 11.10.1993 2) 16.05.1994 - 31.05.1994 3) 07.07.1994	SPI	0.2400	280	0.08580	09.03.1994/0	4 tillers detectable	green material  grain  straw	0.17 <0.05  <0.05  <0.10	20 62  120  120	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002284-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30048/9 0048-93 Germany 51399 Burscheid 1994	Wheat, winter Orestis	1) 03.11.1993 2) 20.06.1994 - 24.06.1994 3) 03.08.1994	SPI	0.2400	300	0.07980	11.01.1994/0	First leaf unfolded	green material  grain  straw	<0.05 <0.05  <0.05  <0.10	111 155  204  204	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg
RA-2054/93 30166/3 0166-93 Germany Versuchsgut Laacherhof, 40789 Monheim 1994	Wheat, winter Konsul	1) 26.10.1993 2) 12.06.1994 - 17.06.1994 3) 25.07.1994	SPI	0.2400	300	0.07980	11.01.1994/0	2 leaves unfolded	green material  grain  straw	0.06 <0.05  <0.05  <0.10	104 146  195  195	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002284-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30256/2 0256-93 France, north 27150 Saussay La Campagne 1994	Wheat, winter Apollo	1) 14.10.1993 2) 08.06.1994 - 18.06.1994 3) 05.08.1994	SPI	0.2400	280	0.08580	09.03.1994/0	3 tillers detectable	green material  grain  straw	0.17 <0.05  <0.05  <0.10	22 84  149  149	(c) SPI: Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002284-01-1)

**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page : Outdoor

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30503/0 0503-93 Germany Versuchsgut Laacherhof, 40789 Monheim 1994	Wheat, winter Contra	1) 28.10.1993 2) 14.06.1994 - 20.06.1994 3) 25.07.1994	SPI	0.2400	300	0.07980	11.01.1994/0	First leaf unfolded	green material  grain  straw	<0.05 <0.05  <0.05  <0.10	104 148  195  195	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002284-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30505/7 0505-93 Netherlands 8256 PT Biddinghuizen 1994	Wheat, winter Estica	1) 02.11.1993 3) 07.08.1994	SPI	0.2400	300	0.07980	06.03.1994/0	2 leaves unfolded	green material  grain  straw	<0.05 <0.05  <0.05  <0.10	72 99  154  154	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg
RA-2054/93 30506/5 0506-93 Netherlands 8255 RT Swifterband 1994	Wheat, winter Ritmo	1) 11.11.1993 3) 10.08.1994	SPI	0.2400	300	0.07980	07.03.1994/0	2 leaves unfolded	green material  grain  straw	<0.05 <0.05  <0.05  <0.10	73 99  156  156	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002284-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT /PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2054/93 30514/6 0514-93 France, north 27700 Frense l'Archeveque 1994	Wheat, winter Scipion	1) 22.10.1993 2) 01.06.1994 - 14.06.1994 3) 03.08.1994	SPI	0.2400	280	0.08580	09.03.1994/0	2 tillers detectable	green material  grain  straw	0.25 <0.05  <0.05  <0.10	22 79  147  147	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-002284-01-1)

2) Northern Europe  
 Supplementary data (Flufenacet + Diflufenican 60 WG)

Report	Crop	Application rate Flufenacet [g as/ha]	Application rate Diflufenican [g as/ha]	Growth stage	Number of trials
RA-2010/94	Barley	240	120*	BBCH 13	1
	Rye	240	120*	BBCH 21	1
	Wheat	240	120*	BBCH 21-25	2
RA-2144/00	Barley	240	120*	BBCH 13	1
	Wheat	240	120*	BBCH 13	1

\*Samples were not analysed for residues of diflufenican

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 20 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2010/94 40044/0 0044-94 Germany Versuchsgut Höfchen, 51399 Burscheid 1994	Barley, winter Loreley	1) 22.09.1994 2) 29.05.1995 - 04.06.1995 3) 13.07.1995	SPI	0.2400	300	0.0800	02.11.1994/0	3 leaves unfolded	green material  grain  straw	<0.05 <0.05  <0.05  <0.1	124 201  253  253	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg    (h) 0.1 mg/kg

cross reference: KCA 6.3.1 (M-004451-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 20 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2010/94 40045/9 0045-94 Germany Versuchsgut Laacherhof, D- 40789 Monheim 1994	Rye, winter Gambit	1) 10.10.1994 2) 24.05.1995 - 30.05.1995 3) 25.07.1995	SPI	0.2400	300	0.0800	21.11.1994/0	Beginning of tillering	green material  grain  straw	0.06 <0.05  <0.05  <0.1	94 172  246  246	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg    (h) 0.1 mg/kg

cross reference: KCA 6.3.1 (M-004451-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 20 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2010/94 40046/7 0046-94 Germany Versuchsgut Höfchen, 51399 Burscheid 1994	Wheat, winter Contra	1) 30.09.1994 2) 10.06.1995 - 18.06.1995 3) 26.07.1995	SPI	0.2400	300	0.0800	21.11.1994/0	5 tillers detectable	green material   grain  straw	<0.05 <0.05  <0.05  <0.10	119 191  247  247	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg    (h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-004451-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 20 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2010/94 40047/5 0047-94 Germany Versuchsgut Laacherhof, 40789 Monheim 1994	Wheat, winter Contra	1) 12.10.1994 2) 10.06.1995 - 17.06.1995 3) 25.07.1995	SPI	0.2400	300	0.0800	21.11.1994/0	Beginning of tillering	green material	0.10 <0.05	133 190	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg   (h) 0.10 mg/kg
									grain	<0.05	246	
									straw	<0.10	246	

cross reference:KCA 6.3.1 (M-004451-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 200 g/L

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2144/00 R 2000 0568 7 0568-00 Germany D-51399 Burscheid, Versuchsgut Höfchen 2000	Barley, winter Theresa	1) 29.09.2000 2) 15.05.2001 - 21.05.2001 3) 23.07.2001	SPI	0.2400	300	0.0800	31.10.2000/0	3 leaves unfolded	grain	<0.05	254	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	254	(h) 0.10 mg/kg
RA-2144/00 R 2000 0566 0 0566-00 France F-37310 Chambourg sur Indre 2000	Wheat, winter Isengrain	1) 14.10.2000 2) 16.05.2001 - 25.05.2001 3) 12.07.2001	SPI	0.2400	300	0.0800	15.11.2000/0	3 leaves unfolded	grain	<0.05	243	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	243	(h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-058156-01-1)

**3) Southern Europe**  
**Supplementary data (Flufenacet + Diflufenican 600 SC)**

Report	Crop	Application rate Flufenacet [g as/ha]	Growth stage	Number of trials
<b>RA-2144/00</b>	<b>Barley</b>	<b>254</b>	<b>BBCH 13</b>	<b>1</b>
	<b>Wheat</b>	<b>240</b>	<b>BBCH 13</b>	<b>1</b>
<b>09-2048</b>	<b>Barley</b>	<b>240</b>	<b>BBCH 13</b>	<b>3</b>
<b>09-2052</b>	<b>Wheat</b>	<b>220-240</b>	<b>BBCH 13-21</b>	<b>4</b>

\*Samples were not analysed for residues of diflufenican

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 200 g/L

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2144/00 R 2000 0570 9 0570-00 Spain E-08289 Veciana 2001	Barley, winter Graphic	1) 29.11.2000 3) 03.07.2001	SPI	0.2540	317.5	0.0800	05.02.2001/0	3 leaves unfolded	grain	<0.05	148	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	0.11	148	(h) 0.10 mg/kg
RA-2144/00 R 2000 0567 9 0567-00 France F-31620 Gargas 2000	Wheat, winter Soissons	1) 11.11.2000 2) 03.05.2001 - 15.05.2001 3) 02.07.2001	SPI	0.2400	300	0.0800	18.12.2000/0	3 leaves unfolded	grain	<0.05	196	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	196	(h) 0.10 mg/kg

cross reference: KCA 6.3.1 (M-058156-01-1)

# Flufenacet

## Volume 3 – B.7 (AS)

### RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 200 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : Flufenacet & Diflufenican SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : flufenacet 400 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
09-2048 09-2048-01 France 31620 Castelnau d'Estretfonds Midi-Pyrenees 2008	Barley, winter Platine	1) 22.10.2008 2) 10.05.2009 - 20.05.2009 3) 23.06.2009 - 05.07.2009	SPI	0.12	300	0.040	10.12.2008/0	3 leaves unfolded	green material  grain  straw	9.4  <0.01  <0.01	0  197  197	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg
09-2048 09-2048-02 France 84490 St Saturnin les Apt Provence-Cote D'Azur 2008	Barley, winter Baraka	1) 22.10.2008 2) 25.04.2009 - 03.05.2009 3) 24.06.2009 - 25.06.2009	SPI	0.12	300	0.040	18.12.2008/0	3 leaves unfolded	green material  grain  straw	13  <0.01  <0.01	0  188  188	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg
09-2048 09-2048-03 France 86170 Ibeil Poitou-Charentes 2008	Barley, winter Esterel	1) 12.10.2008 2) 02.05.2009 - 09.05.2009 3) 20.06.2009 - 26.06.2009	SPI	0.12	300	0.040	01.12.2008/0	3 leaves unfolded	green material  grain  straw	8.8  <0.01  <0.01	0  203  203	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference:KCA 6.3.1 (M-361495-01-1)

# Flufenacet

## Volume 3 – B.7 (AS)

### RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : Flufenacet & Diflufenican SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : diflufenican 200 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment (e)	Portion analysed (a)	Residues (mg/kg)	DALT/ PHI (days) (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
09-2048 09-2048-01 France 31620 Castelnau d'Estretfonds Midi-Pyrenees 2008	Barley, winter Platine	1) 22.10.2008 2) 10.05.2009 - 20.05.2009 3) 23.06.2009 - 05.07.2009	SPI	0.24	300	0.080	10.12.2008/0	3 leaves unfolded	green material  grain  straw	9.2  <0.01  <0.05	0  197  197	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg  (h) 0.05 mg/kg
09-2048 09-2048-02 France 84490 St Saturnin les Apt Provence-Cote D'Azur 2008	Barley, winter Baraka	1) 22.10.2008 2) 25.04.2009 - 03.05.2009 3) 24.06.2009 - 25.06.2009	SPI	0.24	300	0.080	18.12.2008/0	3 leaves unfolded	green material  grain  straw	11  <0.01  0.06	0  188  188	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg  (h) 0.05 mg/kg
09-2048 09-2048-03 France 86170 Ibeil Poitou-Charentes 2008	Barley, winter Esterel	1) 12.10.2008 2) 02.05.2009 - 09.05.2009 3) 20.06.2009 - 26.06.2009	SPI	0.24	300	0.080	01.12.2008/0	3 leaves unfolded	green material  grain  straw	9.5  <0.01  0.06	0  203  203	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg  (h) 0.05 mg/kg

cross reference:KCA 6.3.1 (M-361495-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 200 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : Flufenacet &amp; Diflufenican SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : flufenacet 400 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
09-2052 09-2052-01 France 47550 Boe Aquitaine 2008	Wheat, winter Arlequin	1) 26.10.2008 2) 15.05.2009 - 25.05.2009 3) 01.07.2009 - 15.07.2009	SPI	0.12	300	0.040	11.12.2008/0	3 leaves unfolded	green material  grain  straw	10  <0.01  <0.01	0  209  209	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg
09-2052 09-2052-02 France 26300 Alixan Rhône-Alpes 2009	Wheat, winter Aubusson	1) 15.10.2008 2) 13.05.2009 - 20.05.2009 3) 01.07.2009 - 02.07.2009	SPI	0.12	300	0.040	29.01.2009/0	Beginning of tillering	green material  grain  straw	12  <0.01  0.01	0  153  153	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-363200-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 200 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : Flufenacet &amp; Diflufenican SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : flufenacet 400 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
09-2052 09-2052-03 France 69650 Quincieux Rhône-Alpes 2008	Wheat, winter Aubusson	1) 19.10.2008 2) 15.05.2009 - 25.05.2009 3) 30.06.2009 - 07.07.2009	SPI	0.11	260	0.043	16.12.2008/0	3 leaves unfolded	green material  grain  straw	9.4  <0.01  <0.01	0  196  196	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg
09-2052 09-2052-04 France 79120 Lezay Poitou-Charentes 2008	Wheat, winter Mendel	1) 20.10.2008 2) 14.05.2009 - 26.05.2009 3) 13.07.2009 - 22.07.2009	SPI	0.12	300	0.040	08.12.2008/0	3 leaves unfolded	green material  grain  straw	9.7  <0.01  <0.01	0  220  220	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-363200-02-1)

# Flufenacet

## Volume 3 – B.7 (AS)

### RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : Flufenacet & Diflufenican SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : diflufenican 200 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
09-2052 09-2052-01 France 47550 Boe Aquitaine 2008	Wheat, winter Arlequin	1) 26.10.2008 2) 15.05.2009 - 25.05.2009 3) 01.07.2009 - 15.07.2009	SPI	0.24	300	0.080	11.12.2008/0	3 leaves unfolded	green material  grain  straw	17  0.01  <0.05	0  209  209	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg  (h) 0.05 mg/kg
09-2052 09-2052-02 France 26300 Alixan Rhone-Alpes 2009	Wheat, winter Aubusson	1) 15.10.2008 2) 13.05.2009 - 20.05.2009 3) 01.07.2009 - 02.07.2009	SPI	0.24	300	0.080	29.01.2009/0	Beginning of tillering	green material  grain  straw	22  <0.01  <0.05	0  153  153	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg  (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-363200-02-1)

# Flufenacet

## Volume 3 – B.7 (AS)

### RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 400 g/L

Formulation (e.g. WP) : 600 SC

Commercial product (name) : Flufenacet & Diflufenican SC 600

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : diflufenican 200 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
09-2052 09-2052-03 France 69650 Quincieux Rhône-Alpes 2008	Wheat, winter Aubusson	1) 19.10.2008 2) 15.05.2009 - 25.05.2009 3) 30.06.2009 - 07.07.2009	SPI	0.22	260	0.085	16.12.2008/0	3 leaves unfolded	green material  grain  straw	17  0.05  0.09	0  196  196	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg   (h) 0.05 mg/kg
09-2052 09-2052-04 France 79120 Lezay Poitou-Charentes 2008	Wheat, winter Mendel	1) 20.10.2008 2) 14.05.2009 - 26.05.2009 3) 13.07.2009 - 22.07.2009	SPI	0.24	300	0.080	08.12.2008/0	3 leaves unfolded	green material  grain  straw	24  <0.01  <0.05	0  220  220	(c) SPI:Spraying (g) 01179 (h) 0.01 mg/kg   (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-363200-02-1)

**4) Northern Europe ;  
Supplementary data (Diflufenican + Flufenacet + Flurtamone 360 SC)**

Report	Crop	Application rate Flufenacet [g as/ha]	Growth stage	Number of trials
<b>12-2001</b>	<b>Barley</b>	<b>120</b>	<b>BBCH 22-23</b>	<b>2</b>
	<b>Wheat</b>	<b>120</b>	<b>BBCH 22-25</b>	<b>2</b>
<b>11-2094</b>	<b>Barley</b>	<b>120</b>	<b>BBCH 25</b>	<b>2</b>
<b>11-2095</b>	<b>Wheat</b>	<b>110-120</b>	<b>BBCH 23-27</b>	<b>2</b>

\*Samples were not analysed for residues of diflufenican

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
12-2001 12-2001-01 Germany 49377 Langförden 2011	Barley, winter Meridian	1) 30.09.2011 2) 21.05.2012 - 04.06.2012 3) 05.07.2012 - 25.07.2012	SPI	0.12	300	0.040	22.11.2011/0	3 tillers detectable	green material  whole plant without root  grain  straw	<0.01  <0.01  <0.01  <0.05	170  216  244  244	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)   (h) 0.05 mg/kg
12-2001 12-2001-02 Belgium 6210 Villers-Perwin 2011	Barley, winter Saskia (early 6-rows variety, mid height)	1) 26.09.2011 2) 21.05.2012 - 28.05.2012 3) 16.07.2012 - 22.07.2012	SPI	0.12	300	0.040	09.11.2011/0	2 tillers detectable	green material  whole plant without root  grain  straw	<0.01  <0.01  <0.01  <0.05	181  209  252  252	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)   (h) 0.05 mg/kg

cross reference:KCA 6.3.1 (M-459795-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	DALT/ PHI (days)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL	(d)	(e)	(a)		(f)	
12-2001 12-2001-03 Germany 59457 Werl- Westönnen 2011	Wheat, winter Inspiration	1) 05.10.2011 2) 04.06.2012 - 11.06.2012 3) 15.07.2012 - 23.08.2012	SPI	0.12	300	0.040	14.11.2011/0	2 tillers detectable	green material  whole plant without root  grain  straw	<0.01  <0.01  <0.01  <0.05	192  239  263  263	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)   (h) 0.05 mg/kg
12-2001 12-2001-04 Netherlands 1774 PE Slootdorp 2012	Wheat, winter Taureq winter	1) 10.11.2011 2) 17.06.2012 - 02.07.2012 3) 06.08.2012 - 18.08.2012	SPI	0.12	300	0.040	20.04.2012/0	5 tillers detectable	green material  whole plant without root  grain  straw	<0.01  <0.01  <0.01  <0.05	41  83  112  112	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)   (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-459795-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2094 11-2094-01 Germany 51399 Burscheid 2011	Barley, winter Ketos winter barley	1) 22.09.2011 2) 11.05.2012 - 16.05.2012 3) 15.07.2012 - 31.07.2012	SPI	0.12	300	0.040	04.11.2011/0	5 tillers detectable	green material   whole plant without root  grain  straw	5.4 3.2 2.8 3.1 2.1 <0.01 <0.01  <0.01  <0.01	0 1 3 5 14 181 209  262  262	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg day 0: c=0.011 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2094 11-2094-02 United Kingdom SG8 8SS Cambridge 2012	Barley, winter Carat Winter Barley	1) 26.09.2011 2) 24.05.2012 - 08.06.2012 3) 01.08.2012 - 10.08.2012	SPI	0.12	200	0.060	16.01.2012/0	5 tillers detectable	green material    whole plant without root  grain   straw	16 15 9.3 10 6.4 <0.01 <0.01  <0.01  <0.01	0 1 3 4 14 119 164  203  203	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name : diflufenican 120 g/L

and content) flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2094 11-2094-01 Germany 51399 Burscheid 2011	Barley, winter Ketos winter barley	1) 22.09.2011 2) 11.05.2012 - 16.05.2012 3) 15.07.2012 - 31.07.2012	SPI	0.12	300	0.040	04.11.2011/0	5 tillers detectable	green material    whole plant without root  grain   straw	3.3 1.7 1.6 1.6 1.0 <0.01 0.019  0.017  <0.05	0 1 3 5 14 181 209  262  262	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 181: c=0.016 mg/kg        (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name : diflufenican 120 g/L

and content) flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	DALT/ PHI (days)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL	(d)	(e)	(a)		(f)	
11-2094 11-2094-02 United Kingdom SG8 8SS Cambridge 2012	Barley, winter Carat Winter Barley	1) 26.09.2011 2) 24.05.2012 - 08.06.2012 3) 01.08.2012 - 10.08.2012	SPI	0.12	200	0.060	16.01.2012/0	5 tillers detectable	green material   whole plant without root grain  straw	14 14 2.5 3.9 1.8 0.037 <0.01  <0.01  <0.05	0 1 3 4 14 119 164  203  203	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 0: c=0.022 mg/kg         (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-01 Germany 59457 Werl - Niederbergstrasse 2011	Wheat, winter Akteur	1) 17.10.2010 2) 30.05.2011 - 08.06.2011 3) 20.07.2011 - 25.08.2011	SPI	0.12	300	0.040	06.04.2011/0	5 tillers detectable	green material     whole plant without root  grain   straw	6.9 4.7 4.6 3.6 0.98 <0.01 <0.01  <0.01  <0.01	0 1 3 5 14 51 93  117  117	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-02 Netherlands 1175 KD Lynden (Hoofddorp) 2011	Wheat, winter Tabasco	1) 08.11.2010 2) 15.06.2011 - 21.06.2011 3) 15.08.2011 - 21.08.2011	SPI	0.1104	277	0.0399	18.04.2011/0	3 tillers detectable - 7 tillers detectable	green material     whole plant without root  grain   straw	11 13 12 8.2 2.1 0.13 <0.01  <0.01  <0.01	0 1 3 5 14 43 95  121  121	(c) SPI: Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-01 Germany 59457 Werl - Niederbergstrasse 2011	Wheat, winter Akteur	1) 17.10.2010 2) 30.05.2011 - 08.06.2011 3) 20.07.2011 - 25.08.2011	SPI	0.12	300	0.040	06.04.2011/0	5 tillers detectable	green material      whole plant without root  grain   straw	4.7 3.6 2.5 1.6 0.42 0.020 0.015  0.022  <0.05	0 1 3 5 14 51 93  117  117	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 0: c=0.012 mg/kg         (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-02 Netherlands 1175 KD Lynden (Hoofddorp) 2011	Wheat, winter Tabasco	1) 08.11.2010 2) 15.06.2011 - 21.06.2011 3) 15.08.2011 - 21.08.2011	SPI	0.1104	277	0.0399	18.04.2011/0	3 tillers detectable - 7 tillers detectable	green material     whole plant without root  grain   straw	16 12 5.0 3.8 0.66 0.077 <0.01  <0.01  <0.05	0 1 3 5 14 43 95  121  121	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 0: c=0.022 mg/kg        (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**5) Southern Europe**  
**Supplementary data (Diflufenican + Flufenacet + Flurtamone 360 SC)**

Report	Crop	Application rate Flufenacet [g as/ha]	Growth stage	Number of trials
11-2094	Barley	120	BBCH 25-29	2
11-2095	Wheat	120	BBCH 29-30	2
12-2002	Barley	120	BBCH 23-25	2
	Wheat	120	BBCH 22	1
RA-2153/97	Barley	126	BBCH 13	2
	Wheat	126	BBCH 13	1
RA-2185/98	Wheat	126	BBCH 13	2

\* Samples were not analysed for residues of diflufenican

\*\* Residue data were evaluated on European level for Annex I inclusion of diflufenican. Therefore data for diflufenican are not included.

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/  (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2094 11-2094-03 France 86220 Leugny 2011	Barley, winter Kétos Winter Barley	1) 16.10.2010 2) 06.05.2011 - 12.05.2011 3) 17.06.2011 - 27.06.2011	SPI	0.12	300	0.040	08.03.2011/0	9 or more tillers detectable	green material      whole plant without root grain  straw	7.6 6.8 4.6 3.2 1.7 <0.01 <0.01  <0.01	0 1 3 6 14 55 83  108 108	(c) SPI: Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2094 11-2094-04 Italy 44124 Ferrara 2011	Barley, winter Aldebaran winter variety	1) 13.10.2010 2) 01.05.2011 - 18.05.2011 3) 08.06.2011 - 30.06.2011	SPI	0.12	400	0.030	21.03.2011/0	5 tillers detectable	green material     whole plant without root  grain   straw	4.0 3.4 3.0 2.2 0.51 0.086 0.017  0.01 0.44	0 1 3 5 14 28 50  80 80	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**Volume 3 – B.7 (AS)**

**Active substance** : **flufenacet**

Crop/Crop Group : Cereals

Crop/Crop Group : Cereals

Page :

: 120 g/L

Indoor/outdoor	:	Outdoor
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: 360 SC

Other a.s. in formulation (common name	:	diflufenican 120 g/L
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flurtamone 120 g/L

Residues determined as	:	1-fluoro-1-isopropyl-4-nitro-2-methyl-5-phenyl-1H-imidazole
Residues calculated as	:	total residue flufenacet

cross reference: KCA 6.3.1 (M-460003-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : diflufenican 120 g/L

flurtamone 120 g/L

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2094 11-2094-04 Italy 44124 Ferrara 2011	Barley, winter Aldebaran winter variety	1) 13.10.2010 2) 01.05.2011 - 18.05.2011 3) 08.06.2011 - 30.06.2011	SPI	0.12	400	0.030	21.03.2011/0	5 tillers detectable	green material     whole plant without root  grain   straw	3.0 2.9 1.8 1.2 0.44 0.081 0.044  0.059	0 1 3 5 14 28 50  80  80	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 0: c=0.024 mg/kg        (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-460003-01-1)

**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-03 France 86270 Mairé 2011	Wheat, winter Cezanne	1) 21.10.2010 2) 02.05.2011 - 13.05.2011 3) 01.07.2011 - 10.07.2011	SPI	0.12	300	0.040	08.03.2011/0	9 or more tillers detectable	green material     whole plant without root  grain   straw	16 14 7.1 6.9 2.5 <0.01 <0.01  <0.01  <0.01	0 1 3 6 14 57 90  119  119	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : diflufenican

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : flufenacet 120 g/L  
flurtamone 120 g/L

Residues determined as : diflufenican

Residues calculated as : diflufenican

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-04 Spain 08520 Marata - Les Franqueses 2011	Wheat, winter Moncada; sowing seed production	1) 05.01.2011 2) 25.04.2011 - 05.05.2011 3) 15.06.2011 - 30.06.2011	SPI	0.12	300	0.040	09.03.2011/0	Beginning of stem elongation	green material     whole plant without root  grain   straw	10 12 7.7 2.9 0.95 <0.01 <0.01  <0.01  <0.01	0 1 2 5 14 42 68  103  103	(c) SPI:Spraying (g) 01091/M001 (h) 0.01 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	DALT/ PHI (days)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL	(d)	(e)	(a)		(f)	
11-2095 11-2095-03 France 86270 Mairé 2011	Wheat, winter Cezanne	1) 21.10.2010 2) 02.05.2011 - 13.05.2011 3) 01.07.2011 - 10.07.2011	SPI	0.12	300	0.040	08.03.2011/0	9 or more tillers detectable	green material     whole plant without root grain  straw	12 8.6 4.3 1.9 0.70 0.033 0.017  0.020  <0.05	0 1 3 6 14 57 90  119  119	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 0: c=0.013 mg/kg         (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-2095 11-2095-04 Spain 08520 Marata - Les Franqueses 2011	Wheat, winter Moncada; sowing seed production	1) 05.01.2011 2) 25.04.2011 - 05.05.2011 3) 15.06.2011 - 30.06.2011	SPI	0.12	300	0.040	09.03.2011/0	Beginning of stem elongation	green material      whole plant without root  grain   straw	8.3 8.3 5.1 0.84 0.41 0.071 0.061  0.035  0.05	0 1 2 5 14 42 68  103  103	(c) SPI: Spraying (g) 01100/M002 (h) 0.01 mg/kg day 0: c=0.015 mg/kg          (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-459755-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : diflufenican 120 g/L

flurtamone 120 g/L

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/	Growth stage at last treatment	Portion analysed	Residues (mg/kg)	DALT/ PHI (days)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL	(d)	(e)	(a)		(f)	
12-2002 12-2002-01 France 13103 Saint Etienne du gres 2011	Barley, winter Platine	1) 14.10.2011 2) 20.04.2012 - 27.04.2012 3) 15.06.2012 - 25.06.2012	SPI	0.12	300	0.040	12.12.2011/0	5 tillers detectable	green material  whole plant without root  grain  straw	0.035  0.045  <0.01  0.069	121  155  192  192	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)    (h) 0.05 mg/kg
12-2002 12-2002-02 Italy 37050 Perzacco 2012	Barley, winter Amillis	1) 18.10.2011 2) 01.05.2012 - 08.05.2012 3) 30.06.2012 - 20.07.2012	SPI	0.12	300	0.040	02.03.2012/0	3 tillers detectable	green material  whole plant without root  grain  straw	0.027  0.025  <0.01  <0.05	46  76  105  105	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)    (h) 0.05 mg/kg

cross reference: KCA 6.3.1 (M-459799-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 120 g/L

Formulation (e.g. WP) : 360 SC

Commercial product (name) : DFF &amp; FFA &amp; FLT SC 360

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

 Other a.s. in formulation (common name and content) : diflufenican 120 g/L  
flurtamone 120 g/L

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
12-2002 12-2002-04 Portugal 2005-009 Casais da Narcisa 2011	Wheat, winter Hystar	1) 10.10.2011 2) 20.04.2012 - 04.05.2012 3) 15.06.2012 - 15.07.2012	SPI	0.12	300	0.040	28.11.2011/0	2 tillers detectable	green material  whole plant without root  grain  straw	<0.01  <0.01  <0.01  <0.05	129  185  213  213	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg Storage temperature exceeded, therefore special stability study (S13-02753)  (h) 0.05 mg/kg

cross reference:KCA 6.3.1 (M-459799-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 350 g/kg

Formulation (e.g. WP) : 70 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 70

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 35 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2153/97 70258/7 0258-97 France F-01440 Viriat 1997	Barley, winter Vertige	1) 18.10.1997 2) 08.05.1998 - 18.05.1998 3) 30.06.1998	SPI	0.1260	280	0.0452	27.11.1997/0	3 leaves unfolded	grain	<0.05	215	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg  *other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrently with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw
									straw	<0.05	215	

cross reference: KCA 6.3.1 (M-012486-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 350 g/kg

Formulation (e.g. WP) : 70 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 70

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 35 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2153/97 70731/7 0731-97 France F-01190 St. Benigne 1997	Barley, winter Pastoral	1) 06.10.1997 2) 08.05.1998 - 18.05.1998 3) 24.06.1998	SPI	0.1260	280	0.0452	07.11.1997/0	3 leaves unfolded	grain	<0.05	229	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg  *other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrent with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw
									straw	<0.05	229	

cross reference: KCA 6.3.1 (M-012486-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 350 g/kg

Formulation (e.g. WP) : 70 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 70

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 35 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2153/97 70732/5 0732-97 France F-01190 St. Benigne 1997	Wheat, winter Soissons	1) 22.10.1997 2) 18.05.1998 - 02.06.1998 3) 15.07.1998	SPI	0.1260	280	0.0452	18.12.1997/0	3 leaves unfolded	grain	<0.05	209	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg  *other than stated in the report on the employed residue analysis method, the required method validation conducted prior to and concurrent with the analysis of treated samples allowed for an LOQ of 0.05 mg/kg not only for grain but also for straw
									straw	<0.05	209	

cross reference: KCA 6.3.1 (M-012486-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 350 g/kg

Formulation (e.g. WP) : 70 WG

Commercial product (name) : FOE 5043 &amp; DIFLUFENICAN WG 70

Producer of commercial product : Bayer CropScience AG

Active substance : Flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) : Diflufenican 35 %

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2185/98 R 1998 1726 0 1726-98 France F-01380 Bage-La Ville 1998	Wheat, winter Sideral	1) 24.09.1998 2) 17.05.1999 - 26.05.1999 3) 15.07.1999	SPI	0.1260	280	0.0452	22.10.1998/0	3 leaves unfolded	grain	<0.05	266	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.05	266	
RA-2185/98 R 1998 1727 9 1727-98 France F-01190 Saint Benigne 1998	Wheat, winter Isangrain	1) 15.10.1998 2) 20.05.1999 - 31.05.1999 3) 15.07.1999	SPI	0.1260	280	0.0452	21.12.1998/0	3 leaves unfolded	grain	<0.05	206	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.05	206	

cross reference: KCA 6.3.1 (M-033163-01-1)

- 6) Processing : Wheat and barley  
Supplementary data for flufenacet**

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/P HI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-3401 11-3401-01 United Kingdom CB22 5EU Cambridge 2011	Wheat, winter Robigus (Winter wheat nabim Gp 3)	1) 14.10.2010 2) 08.06.2011 - 18.06.2011 3) 06.01.2012 - 20.01.2012	SPI	0.48	200	0.24	27.03.2011/0	5 tillers detectable	grain bran middlings shorts white flour white bread whole meal wholemeal bread wheat germ	0.10 0.085 0.41  0.28 0.41 0.012 0.046 0.10 0.086 0.11	135 135 135  135 135 135 135 135 135	(c) SPI:Spraying (g) 01100/M001 (h) 0.01 mg/kg

cross reference:KCA 6.5.3 (M-457286-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/P HI (days)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL					(f)	
cont'd: 11-3401 11-3401-01 United Kingdom CB22 5EU Cambridge 2011									starch A  gluten  starch B  gluten feed meal	<0.01  0.091  0.020  0.053	135  135  135  135	

cross reference: KCA 6.5.3 (M-457286-01-1)

**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/P HI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-3401 11-3401-02 Netherlands 1175 KD Lijnden 2011	Wheat, winter Tabasco	1) 08.11.2010 2) 15.06.2011 - 21.06.2011 3) 15.08.2011 - 21.08.2011	SPI	0.48	300	0.16	19.04.2011/0	5 tillers detectable	grain bran middlings shorts white flour white bread whole meal wholemeal bread wheat germ	0.011 0.015 0.067  0.042 0.069 <0.01 0.011 0.017 0.016 0.021	120 120 120  120 120 120 120 120	(c) SPI: Spraying (g) 01100/M001 (h) 0.01 mg/kg

cross reference: KCA 6.5.3 (M-457286-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/P HI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
cont'd: 11-3401 11-3401-02 Netherlands 1175 KD Lijnden 2011									starch A  gluten  starch B  gluten feed meal	<0.01  0.015  <0.01  <0.01	120  120  120  120	

cross reference: KCA 6.5.3 (M-457286-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : FOE 5043 Trifluoro acetamide

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
107840 STF-F3082-94P USA Stilwell, Kansas 1995	Wheat, winter Karl 92	1) 29.09.1994 3) 07.07.1995	SPI	2.016	94	2.1	14.03.1995/0	4-leaf	grain	1.76	115	(c) SPI:Spraying (g) Gould, T.J.; Lemke, V.J.: Bayer Ag Div Report 106406, 11.05.1995 (h) 0.05 mg/kg  Appl 1 +X-77 0.25 %
									bran	3.61	115	
									flour	0.78	115	
									shorts	1.56	115	
									middlings	1.41	115	
									germ	2.28	115	
									aspirated grain fractions	0.86	115	

cross reference: KCA 6.5.3 (M-002403-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-3400 11-3400-01 Germany 49377 Vechta-Langfoerden 2011	Barley, spring Simba	1) 24.03.2011 2) 07.06.2011 - 18.06.2011 3) 10.07.2011 - 21.08.2011	SPI	0.48	300	0.16	27.04.2011/0	5 tillers detectable	malt sprouts	<0.01	116	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg
									brewer's malt	<0.01	116	
									brewer's grain	<0.01	116	
									hops draff	<0.01	116	
									brewer's yeast	<0.01	116	
									beer	<0.01	116	
									distillers grain, fresh	<0.01	116	
									distillers grain, dried	0.012	116	

cross reference: KCA 6.5.3 (M-468736-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page :

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
cont. 11-3400 11-3400-01 Germany 49377 Vechta- Langfoerden 2011									pearl barley rub off pearl barley  grain, stored	0.018  <0.01  <0.01 <0.01 <0.01 <0.01 <0.01	116  116  116 116 116 116 116	

cross reference: KCA 6.5.3 (M-468736-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

Residues determined as : 4-fluoro-N-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
11-3400 11-3400-02 Belgium 6210 Saint-Amand 2011	Barley, spring Quench	1) 22.02.2011 2) 20.05.2011 - 10.06.2011 3) 10.08.2011	SPI	0.48	200	0.24	18.04.2011/0	3 tillers detectable	malt sprouts  brewer's malt  brewer's grain hops draff  brewer's yeast beer  distillers grain, fresh distillers grain, dried pearl barley rub off	<0.01  <0.01  <0.01  <0.01  <0.01  <0.01  0.013  0.021	114  114  114  114  114  114  114	(c) SPI:Spraying (g) 01100/M002 (h) 0.01 mg/kg

cross reference:KCA 6.5.3 (M-468736-02-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim

Country : Germany

Content of active substance (g/kg or g/L) : 600 g/kg

Formulation (e.g. WP) : 60 WG

Commercial product (name) : Flufenacet WG 60

Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet

Crop/Crop Group : Cereals

Page

Indoor/outdoor : Outdoor

Other a.s. in formulation (common name and content) :

 Residues determined as : 4-fluoro-*N*-isopropylaniline

Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Plot Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest 4) Transplanting	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/ PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
cont. 11-3400 11-3400-02 Belgium 6210 Saint-Amand 2011									pearl barley  grain, stored	<0.01  <0.01 <0.01 <0.01 <0.01 <0.01	114  114 114 114 114 114	

cross reference:KCA 6.5.3 (M-468736-02-1)

**7) Field rotational crop studies  
Supplementary data for flufenacet**

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim  
 Country : Germany  
 Content of active substance (g/kg or g/L) : 240 g/kg (A)  
 400 g/L (B)  
 Formulation (e.g. WP) : 41.5 WG (A)  
 500 SC (B)  
 Commercial product (name) : Flufenacet & Metribuzin WG 41.5 (A)  
 FOE 5043 & Diflufenican SC 500 (B)  
 Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet  
 Crop/Crop Group : Cereals  
 Page :  
 Indoor/outdoor : Outdoor  
 Other a.s. in formulation (common name and content) : metribuzin 17.5 % (A)  
 diflufenican 100 g/L (B)  
 Residues determined as : FOE 5043 Trifluoro acetamide  
 Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/06 R 2006 0003 8 0003-06 United Kingdom GB-SG8 85S Great Chishill 2006	Potato Maris Peer (Rotation: 0)	1) 28.04.2006 2) 13.07.2006 - 15.07.2006 3) 01.09.2006 - 15.09.2006	SPI (A)	0.60	300	0.20	03.05.2006/0	End of dormancy				(c) SPI:Spraying
	Wheat, winter Consort (Rotation: 1)	1) 25.09.2006 2) 15.06.2007 - 20.06.2007 3) 15.08.2007 - 25.08.2007	SPI (B)	0.24	300	0.080	27.10.2006/177	3 leaves unfolded	green material	<0.05	179	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	294	(g) 00346 (h) 0.10 mg/kg
									grain	<0.01	294	(g) 00346/E004 (h) 0.01 mg/kg

cross reference: KCA 6.6.2 (M-306269-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim  
 Country : Germany  
 Content of active substance (g/kg or g/L) : 240 g/kg (A)  
 400 g/L (B)  
 Formulation (e.g. WP) : 41.5 WG (A)  
 500 SC (B)  
 Commercial product (name) : Flufenacet & Metribuzin WG 41.5 (A)  
 FOE 5043 & Diflufenican SC 500 (B)  
 Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet  
 Crop/Crop Group : Cereals  
 Page :  
 Indoor/outdoor : Outdoor  
 Other a.s. in formulation (common name and content) : metribuzin 17.5 % (A)  
 diflufenican 100 g/L (B)  
 Residues determined as : FOE 5043 Trifluoro acetamide  
 Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/06 R 2006 0046 1 0046-06 Germany D-51799 Burscheid 2006	Potato Cilena (Rotation: 0)	1) 09.05.2006 2) 12.06.2006 - 28.06.2006 3) 13.09.2006	SPI (A)	0.60	300	0.20	17.05.2006/0	End of dormacy				(c) SPI:Spraying
	Wheat, winter Limes (Rotation: 1)	1) 27.09.2006 2) 17.06.2007 - 21.06.2007 3) 17.07.2007	SPI (B)	0.24	300	0.080	13.10.2006/149	2 leaves unfolded	green material	<0.05	147	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	277	(g) 00346 (h) 0.10 mg/kg
									grain	<0.01	277	(g) 00346/E004 (h) 0.01 mg/kg

cross reference: KCA 6.6.2 (M-306269-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim  
 Country : Germany  
 Content of active substance (g/kg or g/L) : 240 g/kg (A)  
 400 g/L (B)  
 Formulation (e.g. WP) : 41.5 WG (A)  
 500 SC (B)  
 Commercial product (name) : Flufenacet & Metribuzin WG 41.5 (A)  
 FOE 5043 & Diflufenican SC 500 (B)  
 Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet  
 Crop/Crop Group : Cereals  
 Page :  
 Indoor/outdoor : Outdoor  
 Other a.s. in formulation (common name and content) : metribuzin 17.5 % (A)  
 diflufenican 100 g/L (B)  
 Residues determined as : FOE 5043 Trifluoro acetamide  
 Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code  Year of Trial	Commodity / Variety  (a)	Date of 1) Sowing or planting 2) Flowering 3) Harvest  (b)	Method of treatment  (c)	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/PHI (days)  (f)	Remarks
				kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/06 R 2006 0418 1 0418-06 Germany D-49377 Vechta- Langenförden 2006	Potato Cilena (Rotation: 0)	1) 04.05.2006 2) 29.06.2006 - 19.07.2006 3) 18.09.2006 - 22.09.2006	SPI (A)	0.60	300	0.20	16.05.2006/0	Beginning of root formation				(c) SPI:Spraying
	Barley, winter Franziska (Rotation: 1)	1) 09.10.2006 2) 26.05.2007 - 02.06.2007 3) 16.07.2007 - 17.07.2007	SPI (B)	0.24	300	0.080	03.11.2006/171	3 leaves unfolded	green material	<0.05	164	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	255	(g) 00346 (h) 0.10 mg/kg
									grain	<0.01	255	(g) 00346/E004 (h) 0.01 mg/kg

cross reference: KCA 6.6.2 (M-306269-01-1)

**Flufenacet**
**Volume 3 – B.7 (AS)**
**RESIDUE DATA FROM SUPERVISED TRIALS (SUMMARY)**

(Application on agricultural and horticultural crops)

Responsible body for reporting (name and address) : Bayer CropScience AG, Monheim  
 Country : Germany  
 Content of active substance (g/kg or g/L) : 240 g/kg (A)  
 400 g/L (B)  
 Formulation (e.g. WP) : 41.5 WG (A)  
 500 SC (B)  
 Commercial product (name) : Flufenacet & Metribuzin WG 41.5 (A)  
 FOE 5043 & Diflufenican SC 500 (B)  
 Producer of commercial product : Bayer CropScience AG

Active substance : flufenacet  
 Crop/Crop Group : Cereals  
 Page :  
 Indoor/outdoor : Outdoor  
 Other a.s. in formulation (common name and content) : metribuzin 17.5 % (A)  
 diflufenican 100 g/L (B)  
 Residues determined as : FOE 5043 Trifluoro acetamide  
 Residues calculated as : total residue flufenacet

1	2	3	4	5			6	7	8	9	10	11
Study Trial No.; Trial SubID Location incl. postal code	Commodity / Variety	Date of 1) Sowing or planting 2) Flowering 3) Harvest	Method of treatment	Application rate per treatment			Dates of treatment(s)/ Application interval  or no. of treatments and last date/ (d)	Growth stage at last treatment  (e)	Portion analysed  (a)	Residues (mg/kg)	DALT/PHI (days)  (f)	Remarks
Year of Trial	(a)	(b)	(c)	kg a.s./ha	Water (L/ha)	kg a.s./hL						
RA-2020/06 R 2006 0420 3 0420-06 France F-80700 Champien 2006	Potato Pomme Fine (Rotation: 0)	1) 25.04.2006 2) 01.07.2006 - 25.07.2006 3) 15.09.2006 - 30.09.2006	SPI (A)	0.60	300	0.20	05.05.2006/0	Beginning of root formation				(c) SPI:Spraying
	Barley, winter Colibri (Rotation: 1)	1) 10.10.2006 3) 06.07.2007 - 14.07.2007	SPI (B)	0.24	300	0.080	30.11.2006/209	2 tillers detectable	green material	<0.05	97	(c) SPI:Spraying (g) 00346 (h) 0.05 mg/kg
									straw	<0.10	218	(g) 00346 (h) 0.10 mg/kg
									grain	<0.01	218	(g) 00346/E004 (h) 0.01 mg/kg

cross reference: KCA 6.6.2 (M-306269-01-1)